# COMPARATIVE EVALUATION OF THREE DIFFERENT DRYING PROTOCOLS ON INTRATUBULAR PENETRATION AND PUSH OUT BOND STRENGTH OF RESIN BASED SEALER AND CALCIUM SILICATE BASED SEALER- AN IN VITRO STUDY

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# LIST OF ABBREVIATIONS

| Sr.no | Abbreviations | Full form |
|---|---|---|
| 01. | SEM | Scanning Electron Microscope |
| 02. | TEM | Transmission Electron Microscope |
| 03. | CLSM | Confocal Laser Scanning Microscope |
| 04. | EDTA | Ethylene Diamine Tetra-acetic Acid |
| 06. | NaOCl | Sodium Hypochlorite |
| 07. | RDIZ | Resin Dentin Interdiffusion Zone |
| 08. | Min | Minutes |
| 09. | Ml | Milli litres |
| 10. | Ni-Ti | Nickle Titanium |
| 11. | RITC | Rhodamine B IsoThioCynate |
| 12. | OSHA | Occupational Safety and Health Administration |
| 13. | CDC | Centre for Disease Control |
| 14. | RVG | Radio Visio Graphy |
| 15. | CEJ | Cemento-Enamel Junction |
| 16. | WL | Working Length |
| 17. | sec | Seconds |
| 18. | SPSS | Statistical Package for the Social Sciences |
| 19. | µm | Micro meter |
| 20. | ANOVA | Analysis of Variance |
| 21. | HSD | Honest Significant Difference |

| Sr.no | Abbreviations | Full form |
|---|---|---|
| 22. | SD | Standard Deviation |
| 23. | S | Significant |
| 24. | NS | Not Significant |
| 25. | HS | Highly Significant |
| 26. | N | Number of specimens |
| 27. | p-value | Probability of obtaining a test statistic at least as extreme as the one that was actually observed |
| 28. | Max. | Maximum |
| 29. | Min. | Minimum |
| 30. | No. | Number |
| 31. | CI | Confidence Interval |
| 32. | CHX | Chlorhexidine |
| 33. | Mm | Milli Meter |
| 34. | UTM | Universal Testing Machine |
| 35. | AWL | Actual Working Length |

# INTRODUCTION

*"It is through science that we prove, but through intuition that we discover"*

*- Henri Poincare*

In contemporary endodontics, the main objective of root canal treatment is to complement proper shaping and cleaning of the root canal system, 3-dimensional filling with a biologically inert and dimensionally stable material.[1,2] To serve this aim, gutta-percha has been used in combination with various endodontic sealers.[3] Numerous endodontic materials have been developed for complete and impermeable fillings. An ideal root canal sealer should adhere to both root dentin wall and the obturating material.[4,5] They should seal the root canal apically and laterally, and also fill voids and irregularities. These sealers along with obturating materials forms a fluid-tight seal which reduces apical leakage and bacterial contamination, prevents apical periodontitis, and entombs the remaining irritants in the root canal.[6,7]

As the sealer penetrates the dentinal tubules it, provides a fluid-tight seal[8,9] preventing penetration of microorganisms and their toxins.[10] Also, the strong bond between the root canal sealer and the root dentin is essential for maintaining the integrity of the sealer-dentin interface during the preparation of post-spaces and during tooth flexure.

Attentiveness in adhesive endodontics have led to the introduction of different resin-based root canal sealers. Resin-based root canal sealers have conventionally been used, offering the advantages of reduced solubility, tight apical sealing, and micro retention to the root dentin.[11] Many researchers have frequently used AH Plus an epoxy resin–based sealers as a control material in conjunction with gutta-percha (GP) in various root filling techniques.[12] AH Plus is characterized by very good mechanical properties, high radio opacity, less polymerization shrinkage, reduced solubility, and long term dimensional stability on storage. However, its sealing ability remains controversial partly because AH Plus does not bond to gutta-percha.[13] The toxicity of these sealers is reduced after setting; however, they exhibit toxicity when freshly mixed.[14] To overcome the problem of sealing ability and toxicity, new calcium silicate sealers have been developed.

Newly introduced calcium silicate-based sealers focus predominantly on forming a secondary monoblock unit between the core material, sealer, and root canal dentin. BioRoot RCS is a calcium silicate-based sealer, composed mainly of tricalcium silicate and zirconium oxide powder that must be mixed with a liquid containing calcium chloride.

**Jung et al. in 2018**, conducted an in vitro study comparing epoxy resin-based sealer with calcium silicate sealers and observed that BioRoot RCS showed excellent biocompatibility in both the fresh and set states.[14] **Kim et al. in 2019** evaluated and compared AH Plus and Bio Root RCS for dentinal penetration. AH Plus showed the highest sum flouroscence intensity (SFI) than BioRoot RCS in coronal and apical sections.[15]

Quality of adhesion and intra tubular penetration of sealer in root canal dentin is affected by different levels of residual moisture in the root canals.[16,17] Despite the fact that the perception of moisture may vary widely among clinicians, several manufacturers recommend keeping the root canals in a moist state to improve the dentin hybridization of hydrophilic sealers.[18] Since no clear instructions have been provided for achieving such an ideal degree of residual moisture, various drying protocols have been suggested and tested to improve dentinal wettability.

Paper points are one of the most commonly and anciently employed protocol for drying of root canals. **Nagas et al. in 2012** have shown that excessive desiccation may remove the water residing in the dentinal tubules, which may inturn hamper effective penetration of hydrophilic sealers, compromising the quality of adhesion.[19]

Various chemicals including alcohol in different concentrations such as 70% isopropyl alcohol and 95% ethanol have been employed as drying protocol to maintain moist condition in root canals. **Kleber CD. et al in 2014** in an observational in vitro study reported that final rinse with 70% isopropyl alcohol has shown promising results to improve resin–based sealer penetration into the dentinal tubules. However, its effect is still unclear when using calcium silicate-based sealer in the obturation procedure.[20]

Recently introduced Endodontic aspirating tip for the root canal have been specially designed to dry root canals. Roeko Surgitip-endo is an aspirating tip with an innovative design of a multi-directional flexibility, so it can easily be introduced into hard-to-reach root canals without having to bend the Canal Tip. According to manufactures aspiration is highly effective at all angles and visibility is not constricted. However, presently its effect on sealer penetration and bond strength of resin-based sealer and calcium silicate-based sealer has not been evaluated.

Thus, this in vitro study was aimed to investigate the effect of various drying protocol (Paper point, Isopropyl Alcohol and Roeko surgitip-Endo) on Intratubular penetration and Push out bond strength of Resin based sealer and Calcium silicate-based sealer to root dentin.

The null hypothesis was that there is no significant effect on Intratubular penetration and Push out bond strength of Resin based sealer and calcium silicate based after use of various drying protocols (Paper point, Isopropyl Alcohol and Roeko surgitip Endo).

# AIM & OBJECTIVES

## Aim

To investigate the effect of various drying protocol (Paper point, Isopropyl Alcohol and Roeko surgitip-Endo) on Intratubular penetration and its relation to Push out bond strength of Resin based sealer and Calcium silicate-based sealer to root dentin.

## Objectives: -

1. To evaluate Intratubular penetration of Resin based sealer and calcium silicate-based sealer to root dentin after use of various drying protocols (Paper point, Isopropyl alcohol and Roeko surgitip- Endo).

2. To compare Intratubular penetration of Resin based sealer and calcium silicate-based sealer to root dentin after use of various drying protocols (Paper point, Isopropyl alcohol and Roeko surgitip- Endo).

3. To evaluate Push out bond strength of Resin based sealer and calcium silicate-based sealer to root dentin after use of various drying protocol (Paper point, Isopropyl alcohol and Roeko surgitip- Endo).

4. To compare Push out bond strength of Resin based sealer and calcium silicate-based sealer to root dentin after use of various drying protocol (Paper point, Isopropyl alcohol and Roeko surgitip- Endo).

5. To analyze the relationship between Intratubular penetration and Push out bond strength of Resin based sealer and calcium silicate-based sealer after use of Paper point, Isopropyl alcohol and Roeko surgitip- Endo if any.

# REVIEW OF LITERATURE

Various drying protocols and irrigation strategies have been tried in past for effective sealer penetration and bond strength for effective bonding of sealer with root dentin. Therefore, it is of vital importance to know these drying protocols and techniques that various researchers have used and also difficulties encountered over the years for long term survival of sealer to root dentin.

As it is rightly stated by Confucius "Study the past, if you would divine the future."

**Yuichi Kimura et al. 1997**[21] studied the visualization and quantification of dentin structure in thirty extracted teeth using confocal laser scanning microscopy and stated that confocal microscopy is well suited to the observation of dental and material surfaces and to monitoring the effects of various agents and factors on their microstructure.

**Engel et al. 2005**[22] studied sealer penetration and apical microleakage and evaluated the effect of final rinse with either 70% isopropyl alcohol or Peridex in instrumented, smear-free canals. After instrumentation of sixty extracted teeth, smear layer was removed by irrigating with 5ml of 17% EDTA and were randomly divided into three groups group A, 6% NaOCl; group B, 70% isopropyl alcohol and group C Peridex . The results showed no significant differences for microleakage or sealer penetration between the groups that were finally rinsed with 70% isopropyl alcohol, sodium hypochlorite and Peridex. They concluded that final rinse of either 70% isopropyl alcohol or Peridex, can be safely used as a final rinse in nonsurgical root canal therapy without adversely affecting apical microleakage.

**Zemner O et al. 2007**[23] determined the coronal sealing properties of root fillings with methacrylate resin-coated gutta-percha cones (RGPC) and EndoRez (ER) or Resilon and Epiphany (EP) sealer in instrumented root canal that had different levels of moisture, from dry to wet. Specimens were subjected to four different moisture conditions Group I- Ethanol, Group II- Paper Points, Group III- Moist, Group IV- Wet. The results of this study demonstrated that the moisture condition of root canals at the time of obturation and the type of sealer that was used had a significant effect on microleakage. All materials evaluated showed some evidence of dye penetration, however, root canals filled with resin-coated gutta-percha/EndoRez and Resilon/Epiphany demonstrated significantly less leakage ($P>.05$) when moist conditions paper points and luer vacuum adaptor were present.

**Balguerie et al. 2011**[24] studied and assessed tubular adaptation, penetration depth and adaptation to the root canal walls in the apical, middle and coronal third of the root canal of 5 different sealers used in combination with softened gutta -percha cones by Scanning Electron Microscopy (SEM). The 5 different sealer used were Acroseal, Endobtur, Ketac-Endo, RSA and AH Plus. It was observed that the AH Plus showed the best adaptation to the root canal wall, tubular penetration and adaptation to the peritubular dentin, followed by Acroseal. They stated that epoxy resin sealers like AH Plus shows higher adhesion to the dentin and gutta percha and the flow of AH Plus is significantly higher than other sealers tested. The penetration in the dentinal tubules was significantly greater in the coronal and middle of the root canal than the apical part of the root canal.

**Sagsen et al. 2011**[25] assessed the push-out bond strength of two new calcium silicate-based endodontic sealers in the root canals of thirty extracted teeth. After instrumentation and irrigation using 5 mL 2.5% NaOCl between each instrument, and the smear layer removal using 5 mL 17% EDTA, teeth were randomly distributed to three groups (n = 10). The canals were filled with three different sealers using a cold lateral compaction technique: group 1: AH Plus + gutta-percha, group 2: I Root SP + gutta-percha and group 3: MTA Fillapex + gutta-percha. Three horizontal sections were prepared at a thickness of 1 mm ±0.1 in the apical, middle and coronal parts of each root. They stated that I Root SP and AH Plus had significantly higher bond strength values than the MTA Fillapex ($P < 0.05$). Within the limitation of study, they concluded that 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.

**Nagas et al. 2012**[19] evaluated and compared the effects of moisture on the push-out bond strength of different root canal sealers with radicular dentin. Eighty freshly-extracted, straight, single-rooted human teeth were instrumented to 6% # 40 and after irrigation the roots were randomly assigned to the following 4 experimental groups, group 1- Ethanol(dry), group 2- paper point (normal moisture), group 3- moist, group 4- wet. For each moisture condition, the specimens were further divided with respect to the sealer. The sealer used in these study were AH Plus (Dentsply-Tulsa Dental, Tulsa, OK), iRoot SP (Innovative Bioceramix Inc, Vancouver,Canada), MTA Fillapex (Angelus), Epiphany and Resilon. The result showed that the bond strength of calcium silicate-based sealer to root dentin was higher than that of other sealers in all moisture conditions. All the sealer showed higher bond strengths under moisture conditions and lowest bond strength values under the ‘‘wet’’ condition as the hydrophilicity of sealers is never sufficient to displace water in a totally wet root canal, and the resultant entrapment of water droplets between the sealer dentin interface would lead to disruption of the bond. So, they suggested that it may be advantageous to leave the canals slightly moist before filling procedures.

**EL-Maaita et al. 2013**[26] investigated the effect of smear layer removal on the push-out bond strength between different Calcium Silicate based Cements and dentin**.** Eighty anterior extracted teeth were instrumented to size 50/0.05 apically and were randomly divided into 2 major groups: Group A smear layer preserved, and Group B smear layer removed using irrigation with 17% EDTA. Roots within each major group were further divided into 4 subgroups: ProRoot MTA, Bio dentine, Harvard MTA, Gutta percha and AH-plus sealer**.** Three 2-mm-thick slices were obtained from each root at different section levels (coronal, middle, apical) for push out bond strength test analysis. The results showed that the push-out strength values were significantly reduced when the smear layer was removed in all the groups and concluded that the smear layer is important in the formation of interfacial layer and possibly gets actively involved in the mineral interaction between the Calcium silicates sealer and radicular dentin.

**Dias KC et al.** in **2014**[20] in an invitro study investigated the bond strength, interfacial ultrastructure, and tag penetration of AH Plus and 2 self-adhesive methacrylate resin–based sealers applied to smear-free radicular dentin using 70% isopropyl alcohol as the active final rinse. Eighty straight single-rooted maxillary canines were randomly divided into 2 groups according to drying protocol. In group 1, the canals were blot dried with size 60 paper points (Dentsply Maillefer), in group 2, the canals were irrigated with 70% isopropyl alcohol. The alcohol was left in the canal for 5 seconds and immediately aspirated. They concluded that overall, removal of the smear layer followed by a drying protocol using 70% isopropyl alcohol before canal obturation improved bond strength and penetration of the sealers into dentinal tubules of the root.

**Tasdemir et al. 2014**[27] assessed the influence of various canal-drying techniques on the push-out bond strength between radicular dentine and MTA Fillapex and iRoot SP sealers without the gutta-percha core material. Sixty single rooted teeth were randomly assigned to 1 of 4 groups. In Group 1 excess fluid in the canals was removed using a Luer vacuum adapter, In Group 2 the canals were dried with a single paper point, Group 3 the canals were dried with 3–5 paper points, and in Group 4 the canals were rinsed with 95% ethanol and then dried with 3–5 paper points. The results showed that canal-drying technique influenced the adhesive bond strength between calcium silicate-based root canal sealers and the root canal wall. Canals dried with only 1 paper point and obturated with the iRoot SP sealer showed significantly higher bond strengths to the root canal wall compared to all other subgroups suggesting that it may be advantageous to leave canals slightly moist before obturating with calcium silicate-based sealers.

**Filho et al. 2014**[28] evaluated the push out bond strength of three root canal sealer, a mineral trioxide aggregate (MTA)-based sealer (MTA Fillapex®), an epoxy resin-based sealer (AH Plus®), and a zinc oxide eugenol-based sealer (EndoFill®) to root dentin. The Results showed that AH Plus presented significantly higher bond strengths than the other sealers, while MTA Fillapex showed the lowest bond strengths and concluded that EndoFill® sealer and MTA FillApex® core combination was not superior to AH Plus® sealer and gutta-percha core combination.

**Oliveira et al. 2014**[29] in an invitro study evaluated and compared the push-out bond strength of MTA Fillapex (Angelus, Londrina, Brazil), iRoot SP (Innovative BioCeramix Inc., Vancouver, Canada) and AH Plus to the dentine walls of root canals. The results showed that AH Plus had significantly higher bond strength than both MTA Fillapex and iRoot SP. They concluded that the adhesion to root dentine associated with newer calcium silicate-based sealers was compromised even in a well-monitored laboratory conditions when compared to AH Plus sealer.

**Silva et al. 2015**[30] evaluated the root canal filling and dentinal tubule penetration of MTA Fillapex in comparison to AH Plus, Pulp Canal Sealer EWT and Sealapex by using stereomicroscope and confocal laser microscopy. Based on the findings obtained in the study they concluded that the AH Plus, Pulp Canal Sealer EWT and Sealapex were found to have a good and similar performance regarding filling material adaptation to the root canal walls, except for MTA Fillapex, which showed more failures at 4 and 6 mm from the root apex. These sealers penetrated deeply into the dentinal tubules, except for the Pulp Canal Sealer EWT, which had a shorter penetration than MTA Fillapex and AH Plus at 4 and 6 mm from the root apex, respectively. But, none of the sealer was able to penetrate the whole extension of the dentinal tubules.

**Paula et al. 2016**[31] carried out an invitro study and evaluated the influence of different drying protocols (DPs) of root canal on the apical sealing (AS) and bond strength (BS) of epoxy resin-based sealers (AH Plus®), with calcium hydroxide (Sealapex®) and calcium silicate (MTA Fillapex®), based on Fluid Filtration and Pushout Bond strength tests. They concluded that the drying protocol did not influence the apical sealing of different root canal sealers. But, the highest bond strength values were observed in specimens dried with Isopropyl alcohol, compared with Ethanol and EndoVac.

**Thiruvenkadam G et al. 2016**[32] evaluated the efficacy of 95% ethanol as final drying agent in primary teeth before obturation and assessed using spiral computed tomography (CT). Results of spiral computed tomography images showed primary root canals dried with 95% ethanol manifested better obturation than those dried with paper points.

**Razmi H et al. 2016**[33] carried out an invitro study and evaluated the effect of canal dryness on the push-out bond strength of two resin sealers (AH-Plus and Adseal) and a bioceramic sealer (Endosequence BC sealer) after canal irrigation with sodium hypochlorite (NaOCl) and chlorhexidine (CHX). The results showed that the bond strength of Adseal was not affected by the canal condition or irrigation type.AH-Plus group was not affected by the irrigant type, although the highest bond strength was seen in dry canals. For Endosequence BC sealer, the canal conditions did not affect the bond strength, however, CHX reduced the bond strength.

**Sungur et al. 2016**[34] studied the push-out bond strength and dentinal tubule penetration of tertiary monoblock units consisting of resin- and glass ionomer-based sealers used with coated Gutta Percha(GP), to a secondary monoblock unit consisting of a resin-based sealer used with conventional GP. The teeth were divided into 4 groups corresponding to the sealer and gutta percha used: Group 1, EndoRez sealer and a size 40/0.04 EndoRez point; Group 2, Activ GP sealer and a size 40/0.04 Activ GP point; Group 3, Smartpaste sealer and a size 40/0.04 Smartpoint point; Group 4, AH 26 sealer (Dentsply de Trey, Konstanz,Germany) and a size 40/0.04 GP point. The push out bond strength test showed that the use of coated core materials with resin- and glass ionomer- based sealers did not enhance the bond strength of the root canal obturation material. The confocal laser scanning microscope showed no significant difference amongst the percentage of sealer penetration. SmartSeal showed the least area and depth of tubule penetration while no difference was found amongst the others. Within the limitations of the study, they concluded that dentinal tubule penetration has limited effect on push-out bond strength of the root canal sealers.

**Madhuri et al. 2016**[35] carried out an invitro study on 48 extracted sound anterior teeth to compare the bond strength of four different endodontic sealers to root dentin, that is, Bioceramic sealer (Endosequence), MTA-based sealer (MTA Fill apex), epoxy resin-based sealer (MM-Seal), and dual cure resin-based sealer (Hybrid Root Sealer) through push-out bond strength test. The results showed that Endosequence BC (Bio-ceramic Sealer) showed the highest push-out bond strength among all the groups followed by MM seal(Epoxy resin based sealer) ,Hybrid seal

(Dual cure resin based sealer) and lowest bond strength was observed in MTA Fill apex.

**Piazza et al. 2017**[36], analysed the influence of humidity on the intra-tubular penetration, bond strength and type of failure mode associated with endodontic sealers. Sixty human mandibular premolar teeth were randomly divided into 4 groups Group 1 - AH plus /moist, Group 2 - AH plus/dry, Group 3 - MTA Filapex /moist and Group 4 - MTA Fillapex /dry. They concluded that humid conditions did not influence the intra-tubular penetration, bond strength and failure mode of AH Plus and MTA Fillapex sealers. Consequently, MTA Fillapex exhibited higher intra-tubular penetration values when compared to AH Plus, irrespective of the moisture conditions. Moreover, AH Plus showed bond strength values higher than MTA Fillapex, and was not being influenced by the moisture conditions. The failure mode analysis identified a majority of cohesive failures.

**Wiesse P et al. 2017**[37] investigated the effect of ultrasonic and sonic activation of two root canal sealers (AH Plus and MTA Fillapex) on interfacial adaptation and push -out bond strength (BS) to root canal dentine. They proposed that ultrasonic activation showed higher bond strength, greater penetration into dentinal tubules, greater quantity, density, and length of tags, and better interfacial adaptation of sealers to canal walls than sonic activation and no activation techniques. AH Plus and MTA Fillapex sealers revealed similar patterns of penetration into the dentinal tubules and interfacial adaptation to root dentine, but AH Plus had higher bond strength than MTA Fillapex irrespective of the type of activation.

**Wang et al. 2018**[38] observed the effects of the bioceramic sealer iRoot SP and

AH Plus on root canal filling density, root canal adaptation, and sealer penetration into dentinal tubules when both single cone and warm vertical techniques were used. Within the limitation of study, they concluded that the bioceramic sealer (iRoot SP) had sufficient filling quality and better dentinal tubules penetration regardless of the filling technique used. The better dentinal tubule penetration of iRoot SP combined with its good bioactivity helped to improve the seal of root canal systems as compared to AH Plus sealer.

**Turker et al. 2018**[39] evaluated and compared the effect of smear layer on the penetration depth and push-out bond strength of various root canal sealers in ninety extracted human mandibular premolars. These samples were randomly assigned into 2 groups: smear layer preserved and smear layer removed. Then the roots were further divided into 3 subgroups according to the sealer used: AH 26, BioRoot RCS and MTA Plus. Within the limitations of this in vitro study, they concluded that Smear layer removal adversely affects the adhesion of MTA Plus; however, the same did not hold for AH26 and BioRoot RCS. Additionally, smear layer removal or preservation did not affect the penetration depth and percentage of any root canal sealer. Dentinal tubule penetration had limited effect on the push-out bond strength of the root canal sealers.

**Donnermeyer D et al. 2018**[40] evaluated and compared the push out bond strength of calcium silicate-based sealers with an epoxy resin-based sealer in 80 extracted human mandibular premolars. The samples were randomly divided into four experimental groups. Group A: Total Fill BC Sealer; Group B: Endo CPM Sealer; Group C: BioRoot RCS; control Group D: AH Plus. Samples were sectioned and evaluated for dislodgement resistance and mode of failure. Within the limitations of this study, the following conclusions were drawn; The push-out bond strength of the investigated calcium silicate-based sealers was lower than the push-out bond strength of AH Plus. Total FillBC showed the highest push-out bond strength of the calcium silicate-based sealers followed by BioRoot RCS and Endo CPM Sealer respectively.

**Piai et al. 2018**[41] evaluated the penetrability of a new epoxy-based sealer (Sealer Plus - MK Life, Porto Alegre, RS, Brasil) into dentinal tubules compared to the gold standard sealer (AH Plus - Dentsply Maillefer, Ballaigues, Suiça). Twenty teeth were divided into 2 groups and instrumented using Ni-Ti rotary files, irrigated with NaOCl + EDTA and obturated with cold lateral condensation (CLC). The roots were transversally cleaved under water cooling at 2, 4, and 6 mm from the apex. They concluded that root canal level affected the penetration of the sealer, but no statistically significant differences were found between the two experimental groups ($p > .05$). Sealer plus presented similar dentinal penetration and perimeter integrity to the gold standard AH Plus sealer.

**Dabaj P et al. 2018**[42] evaluated and compared the push-out bond strengths of root canals obturated with Endosequence BC Sealer, along with the thermo-plasticized injectable technique, while comparing it against AH Plus, and cold lateral compaction technique. They results showed that AH Plus had significantly higher push-out bond strength amongst all experimental groups, regardless of the obturation techniques used and also observed that thermo-plasticized injectable technique with Calamus Flow - Delivery - System lowered the bond strengths of the sealers, especially Endosequence - BC - Sealer. Therefore, this technique is not recommended to calcium-silicate-based sealers.

**Hachem et al. 2019**[43] assessed the dentinal tubule penetration of three different sealers, AH Plus, BC Sealer and a novel tricalcium silicate sealer (NTS) to root dentin. 96 extracted human maxillary central incisors were endodontically treated and randomly assigned in three groups, Group1- AH Plus, group2- BC Sealer and group3- NTS. Confocal laser scanning microscopic analysis was performed to examine the depth of penetration of sealers. The results showed that the maximum and mean penetration depths were significantly higher at 5 mm compared to 1 mm from the apex in the AH Plus, BC Sealer and NTS groups. Within the study limitations, they concluded that the BC Sealer and novel tricalcium silicate sealer (NTS) demonstrated better dentinal tubule penetration results compared to AH Plus.

**Alsubait et al. 2019**[44] in their invitro study assessed the antibacterial activity of BioRoot RCS in comparison with the Totalfill BC and AH Plus sealers against E. faecalis biofilms in dentinal tubules using Confocal Laser Scanning Microscope (CLSM). Sixty specimens were randomly divided into four groups: AH Plus, BioRoot RCS, Totalfill BC sealer, and no sealer. The specimens were incubated for 1, 7, and 30 days, stained and each disc were scanned. The CLSM observation analysis confirmed the presence of homogeneous E. faecalis biofilms on the dentin surfaces, with dense penetration into the dentinal tubules after a 3-week incubation period. Within the limitations of the study, the following conclusions were drawn: o Calcium silicate-based root canal sealers exerted antimicrobial effects against E. faecalis biofilms. The antibacterial activity of BioRoot RCS was significantly higher than that of the Totalfill BC and AH Plus sealers after 30 days of exposure.

**Khurana et al. 2019**[45], Carried out a research that aimed to evaluate and compare the push-out bond strength and failure analysis of an epoxy-based root canal sealer AH Plus, a bioceramic-based root canal sealer EndoSequence BC, and a calcium silicate-based root canal sealer MTA Fillapex applied to smear free root dentin using paper points and 70% isopropyl alcohol drying. They concluded that 70% isopropyl alcohol drying improved the bond strength of the root canal sealers with the dentinal tubules better than the ideal paper point drying. The AH/GP group showed lower bond strength than EndoSequence BC /GP ($p < 0.05$) but higher than MTA Fillapex /GP.

**Eymirli A et al. 2019**[46] conducted a research to evaluate the penetration of a tricalcium silicate–based endodontic sealer into dentinal tubules without a core material or with .02 or .04 tapered bioceramic gutta-percha points and compared the time required to remove the root fillings. Thirty specimens were assigned into 3 subgroups. In group 1, the root canals obturated with EndoSequence BC Sealer alone (sealer). In groups 2 and 3, the canals were obturated with BC Sealer in conjunction with #35 .02 taper and #35 .04 taper BC Points, respectively. The results showed that significantly greater sealer penetration and sealer-penetrated area was achieved when the sealer was used with a .04 gutta point, whereas there was no difference between the sealer and .02 gutta point groups. They concluded that use of a matched-taper bioceramic gutta-percha point enhanced the dentinal tubule penetration of the tested tricalcium silicate–based sealer.

**Kim et al. 2019**[15], in an invitro study compared the penetration ability of calcium silicate root canal sealers and conventional resin-based sealer using confocal laser scanning microscopy (CLSM). Within the limitation of study, they concluded that

- The highest sum fluorescence intensity (SFI) level was found in the coronal third, and the lowest in the apical third in all experimental groups.
- AH Plus showed the highest SFI in apical and coronal areas, whereas the BioRoot RCS group showed a relatively higher intensity level in the middle area, similar to AH Plus.
- The maximum sealer penetration distance was higher at the apical third in the AH Plus group compared with BioRoot RCS and Endoseal MTA.

**Srivastava A et al. 2020**[47], carried out an in vitro study to compare the push-out bond strength of root canal space filled with AH Plus and BioRoot RCS after using different irrigants. Eighty extracted premolar teeth were randomly divided into two groups with respect to sealers and further subdivided into four subgroups based upon final irrigant used. The irrigant used were Saline, 5.25% NaOCl + 17%EDTA , 17%EDTA + 2% Chlorhexidine, 17% EDTA + 3% Polyphenol Green Tea Extract. They concluded that BioRoot RCS showed significantly higher bond strength compared to AH Plus. 17% EDTA + green tea showed significantly higher bond strength compared to other irrigants. When irrigant, sealer effect, and their interactions were analyzed, significantly higher bond strength was observed with BioRoot RCS when 17% EDTA + green tea was used as an irrigant as compared to AH Plus.

# MATERIAL AND METHOD

Sixty freshly extracted human mandibular first premolars with single root and single canal were selected for the study. Presence of single canal was assessed by taking bucco-lingual and mesio-distal radiographs. The teeth were cleaned, disinfected and stored as per the recommendations and guidelines laid down by OSHA and CDC (2003 report 17).[48] The selected teeth were stored in phosphate buffer saline solution (Severn, Biotech).[49]

Approval from the Institutional ethics committee was taken for the study.

## METHOD OF SELECTION OF STUDY SUBJECT:

## INCLUSION CRITERIA:

1. Sound Mandibular First Premolars with single canal.

2. Teeth extracted either for orthodontic or periodontal purpose will be selected.

## EXCLUSION CRITERIA:

1. Teeth with caries, trauma, fracture or other defects such as root calcification, root resorption.
2. Teeth with incompletely formed apices.
3. Teeth with developmental anomalies.
4. Teeth with severe curvatures.

## ARMAMENTARIUM:

### Instruments and Equipment:

- Straight probe (GDC, India) (PLATE-I)
- Explorer (GDC, India) (PLATE-I)
- Pair of Tweezers (GDC, India) (PLATE-I)
- Excavator (GDC, India) (PLATE-I)
- Hand Scaler (Satelec P5 Newtron Worktop Scaler, Satelec Acteon)
- Digital Vernier calliper (WorkZone Hand Tools, Germany) (PLATE-I)
- Cotton holder (GDC, India) (PLATE-I)
- Waste receiver (GDC, India) (PLATE-I)
- Mixing spatula (PLATE-II)
- Mixing pad (PLATE-II)
- Endodontic Microbrush (PLATE-II)
- Mini Endo Block (DENTSPLY, Maillefer, Switzerland) (PLATE-II)
- Straight hand piece (NSK, Japan)) (PLATE-II)
- Double sided diamond disc (DFS, Germany) (PLATE-II)

- X-Smart Endomotor (DENTSPLY, Maillefer, Switzerland) (PLATE-III)
- Digital Radiovisiography System (Kodak 5100 RVG, France)
- Gates Glidden drills (MANI, Japan) (PLATE-III)
- Standard 2% K & H files # 10-80 (MANI, Japan) (PLATE-III)
- Reamers (MANI, Japan) (PLATE-IV)
- ProTaper Universal rotary files. (DENTSPLY,Maillefer, Switzerland) (PLATE-IV)
- Lentulospirals (MANI, Japan) (PLATE-IV)
- Roeko Surgi-tip Endo (Coltene, Switzerland) (PLATE-V)
- Calamus Dual 3D Obturation System (DENTSPLY,Maillefer, Switzerland) (PLATE-V)
- Precision cutting saw (IsoMet 5000, Buehler, Germany) (PLATE-XIII)
- Grinder & polisher (Buehler, Germany) (PLATE-XIII)
- Confocal laser scanning microscope (ZEISS with LSM Software ZEN 2007) (PLATE-XIII)
- Universal testing machine (ACME Engineers, Model no. UNITEST-10) (PLATE-XIII)

## Materials:

- Root canal irrigation solutions (PLATE-VI)
  - Sodium hypochlorite (NaOCl) (Hyposept UPS Hygienes, India)
  - Normal saline (0.9 % w/v, Nirlife, India)
- 5ml syringe with 24gauge needle (Nirlife, India) (PLATE-VI)
- RC Help (Prime Dental Products, India) (PLATE-VI)

- Paper points (Dentsply, Maillefer, Switzerland) (PLATE-VII)
- 70% Isopropyl Alcohol (Cerkamed, Poland) (PLATE-VII)
- Rhodamine B dye (Loba Chemie, India) (PLATE-VII)
- Bio Root root canal sealer (Septodent, France) (PLATE-VIII)
- AH Plus root canal sealer (DENTSPLY, Maillefer, Switzerland) (PLATE-VIII)
- Gutta Percha points (DENTSPLY, Maillefer, Switzerland) (PLATE-VIII)

## SAMPLE PREPARATION

All teeth were decoronated 15±1 mm from the apex under copious water irrigation with a double-sided diamond disc (DFS, Germany) to obtain a standardized length of 15mm. After decoronation, the coronal thirds of the canal were enlarged using Gates Glidden drills (Mani, Japan) using sizes 1- 3 in a descending order. The working lengths (WL) were visually established by subtracting 1 mm from the lengths of a size 10 K-file (Mani, Japan) when its tip appeared at the apical foramen. All roots were shaped uniformly at full working lengths with Pro-Taper Universal rotary files to size #40, 0.06 taper using the X-Smart-Endo-motor (Dentsply, Maillefer, Switzerland) in a crown down technique. The root canals were irrigated with 10 mL 5.25% sodium hypochlorite (NaOCl) between each file size using a 30-gauge side vented needle.

After preparation, the root canals were irrigated with 1 ml of 17% EDTA for 1minute followed by rinsing with 10ml distilled water to remove all chemicals. Flooding of distilled water was confirmed by visual inspection at the coronal access and by extrusion through the apical foramen.

## DISTRIBUTION OF STUDY GROUPS:

The sample will be randomly assigned to the following 3 experimental groups to test the effect of various drying protocol: -

| Group | Sample Distribution | No. of Samples |
|---|---|---|
| **Group I** | Canal drying using paper point (Control group) | 20 |
| **Group II** | Canal drying using 70% Isopropyl Alcohol | 20 |
| **Group III** | Canal drying using Roeko surgitip-Endo | 20 |

In group I, the canals were blot dried with size 40 paper points (Dentsply Maillefer, Switzerland) until complete dryness of the last point was confirmed visually.

In group II, after the removal of excess normal saline with size 40 paper points, as in group I, the canals were filled with 1ml of 70% isopropyl alcohol (Cerkamed, Poland). The alcohol was left in the canal for 5 seconds and immediately aspirated with a capillary tip at a low vacuum with a gentle up-and down motion for 5 seconds.

In group III - The root canals were dried with a Roeko Surgitip Endo (Coltene, Switzerland) for 5 seconds. The adapter was operated at low vacuum with gentle up and down motion followed by drying with 1 single paper point for 1 second.

From each drying protocol group the samples were further divided equally, according to the sealer used: -

| Groups | Subgroups | No. of Samples |
|---|---|---|
| **Group I** Canal drying using paper point. | **SUB GROUP Ia :-** Resin based sealer. | 10 |
| | **SUB GROUP Ib:-** Calcium silicate based sealer. | 10 |
| **Group II** Canal drying using 70%Isopropyl Alcohol | **SUB GROUP IIa:-** Resin based sealer. | 10 |
| | **SUB GROUP IIb:-** Calcium silicate based sealer | 10 |
| **Group III** Canal drying using Roeko surgitip-Endo | **SUB GROUP IIIa:-** Resin based sealer. | 10 |
| | **SUB GROUP IIIb:-** Calcium silicate based sealer | 10 |

Master Cone Gutta Percha was selected and snug fit was checked. The sealers, were mixed with rhodamine B isothiocyanate dye and introduced into the canal orifice with a Lentulo spiral (Dentsply, Maillefer, Switzerland) rotated at 500 rpm in a clockwise direction with a slow-speed handpiece inserted up to 1 mm short of the WL and obturated with thermoplasticzed Obturation technique. After complete setting of both sealer, all samples were stored at 37 °C and 100% relative humidity for 1 week.

## SECTIONING OF TOOTH:

The root sample were sectioned with a microtome precision saw (Isomet, Beuhler, Germany) at 1, 4 and 7 mm below the cemento-enamel junction (CEJ). The resulting sections of each tooth were 1±0.1mm thick. Each section represented the coronal, middle and apical part of root canal. The sectioned surfaces were polished with a series of silicon carbide abrasive papers (upto 2400 grit) using running tap water as a lubricant on MetaServ 2000 Grinder polisher machine. (Buehler, Germany). The samples were kept humid during the whole study in humidifier.

## METHODS OF MEASUREMENTS:

### Two parameters were measured:

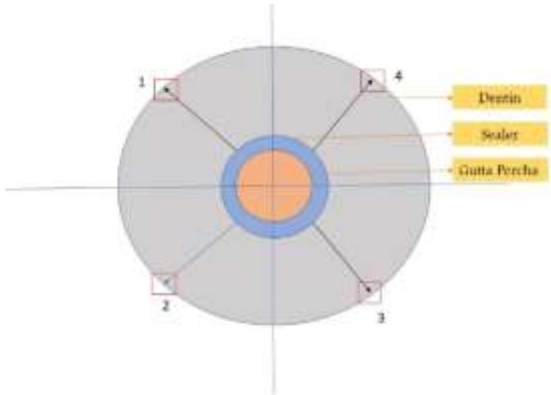
i. Intratubular penetration of Sealers into root dentin.

ii. Push-out bond strength of Sealers to root dentin.

### Intratubular penetration of sealers into root dentin

Confocal Laser Scanning Microscopy (CLSM) was performed with a ‘ZEISS Microscope’ with LSM Software ZEN 2007. An Ar/Kr mixed gas laser was used as the light source. Excitation light had a wavelength maximum at 568 nm. The intensity of the excitation light as well as the amplification of the photomultiplier was kept constant during the investigation period. CLSM images were recorded in fluorescent mode. The detected light was conducted through a 590 nm long-pass filter, thus, fluorescent light emitted from the specimen was discriminated from reflected and scattered light. The visualized layer was selected 10 µm below the sample surface and images were recorded with an oil immersion objective (40x, numerical aperture 1.25).

The size of the images recorded was 62.5 x 62.5 µm2, and the resolution was 512 x 512 pixel.




Images were recorded at four standardized areas of each sample. In order to quantify the depth of penetration, the measurements were performed at four different locations on each image, and a mean was calculated.

## PUSH-OUT BOND TEST:

To evaluate the push-out bond strength same sample were subjected under Universal testing machine (ACME Engineers, India. Model No. UNITEST-10, Accuracy of the machine: ±1%) by applying an axial load to the sealer at a Crosshead speed: 1 mm/minute. The maximum failure load was recorded in Newtons (N) and Converted into megapascals (MPa) using following formula in accordance with **Ferrari et al (2009):**

### Formula for Push out bond strength (MPa) =

$$\frac{\textbf{Push out Load (N)}}{\textbf{Area of bonded interface (sq/mm)}}$$

Where, Area of bonded interface (sq/mm) = $2\pi rh$

$\pi$ = 3.1416,

r = Radius of perforated cross section,

h = Height of perforation

The data was collected and tabulated using an excel sheet (Microsoft Office 2010). This data was then subjected to statistical analysis using a licensed version of SPSS 20.0 (IBM Corp).

## ALGORITHM FOR METHODOLOGY

Extracted human permanent Mandibular First Premolars

↓

Cleaned with Ultrasonic Scaler and Radiograph taken

↓

Decoronation followed by enlargement of coronal third of canal using Gates Glidden Drills

↓

Working Length was determined

↓

Biomechanical Preparation using ProTaper Universal rotary files to size #40, 0.06 taper

↓

Depending upon the drying protocol, samples were divided into 3 different groups (n=20)

- Group I Paper Point (Control)
  - Bio Root Sealer
  - AH Plus Sealer
- Group II Iso Propyl Alcohol
  - Bio Root Sealer
  - AH Plus Sealer
- Group III Roeko – Surgi tip- Endo
  - Bio Root Sealer
  - AH Plus Sealer

Obturation done using Thermoplasticized obturation technique

↓

Samples were sectioned at 1,4,7 mm from CEJ representing the coronal, middle and apical section respectively.

↓

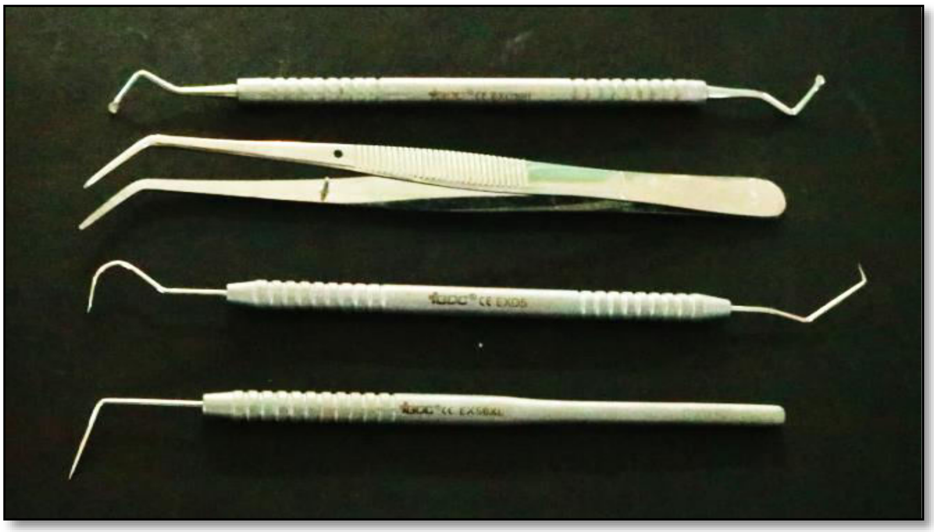
Confocal laser scanning microscopic analysis for depth of penetration of sealers checked

↓

Universal testing machine for push-out bond strength analysis

PLATE -I

# ARMAMENTARIUM



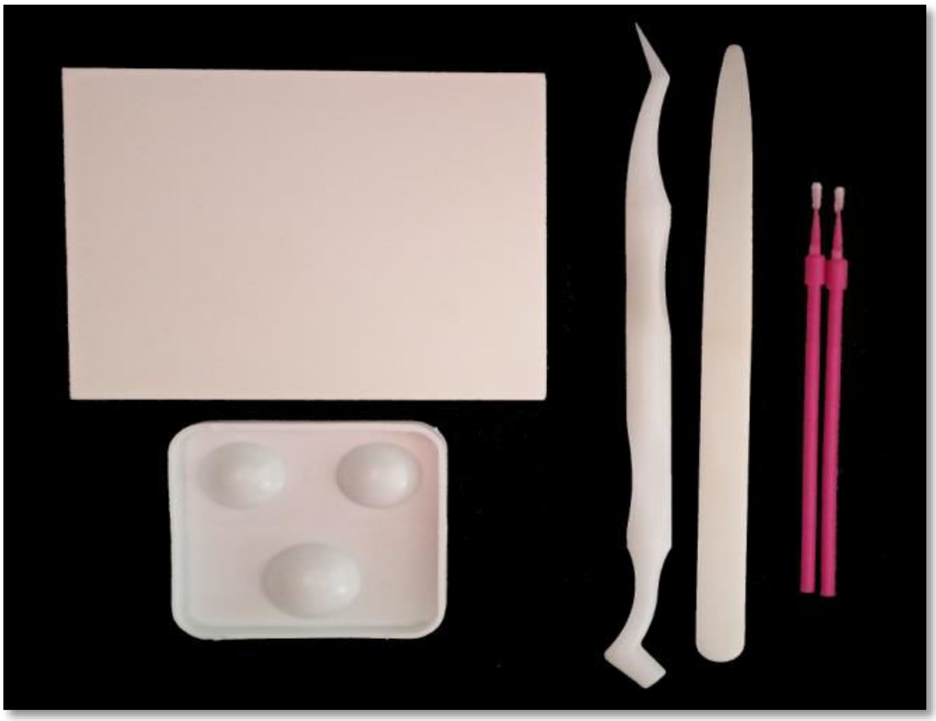
**Hand Instruments** (GDC,India)

**Cotton Holder & Waste receiver** (GDC,India)


**Digital Vernier Caliper** (Workzone tools,Germany)

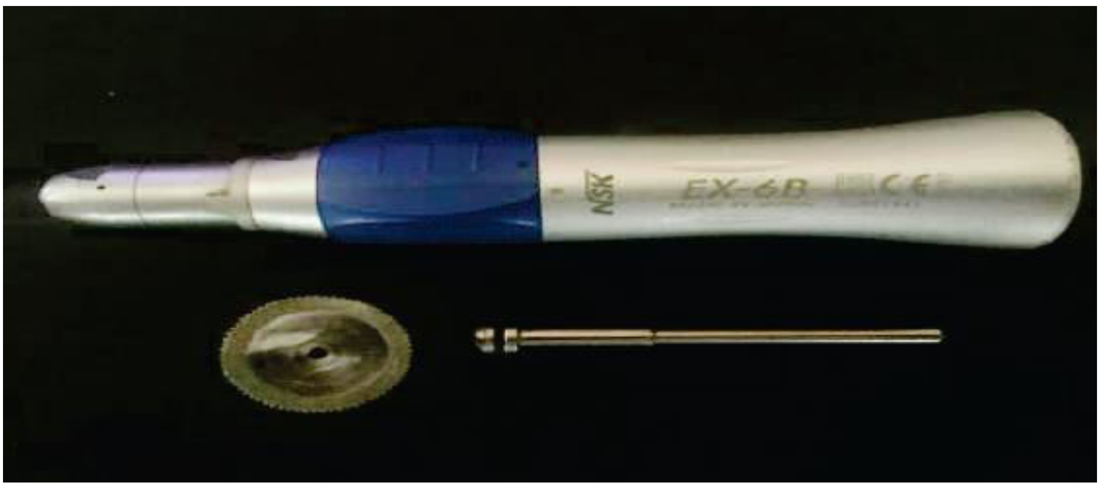
PLATE -II

# ARMAMENTARIUM



**Mixing pad, Spatula, Microbrushes, Dispenser**


**Endobloc** (DENTSPLY, Maillefer, Switzerland)



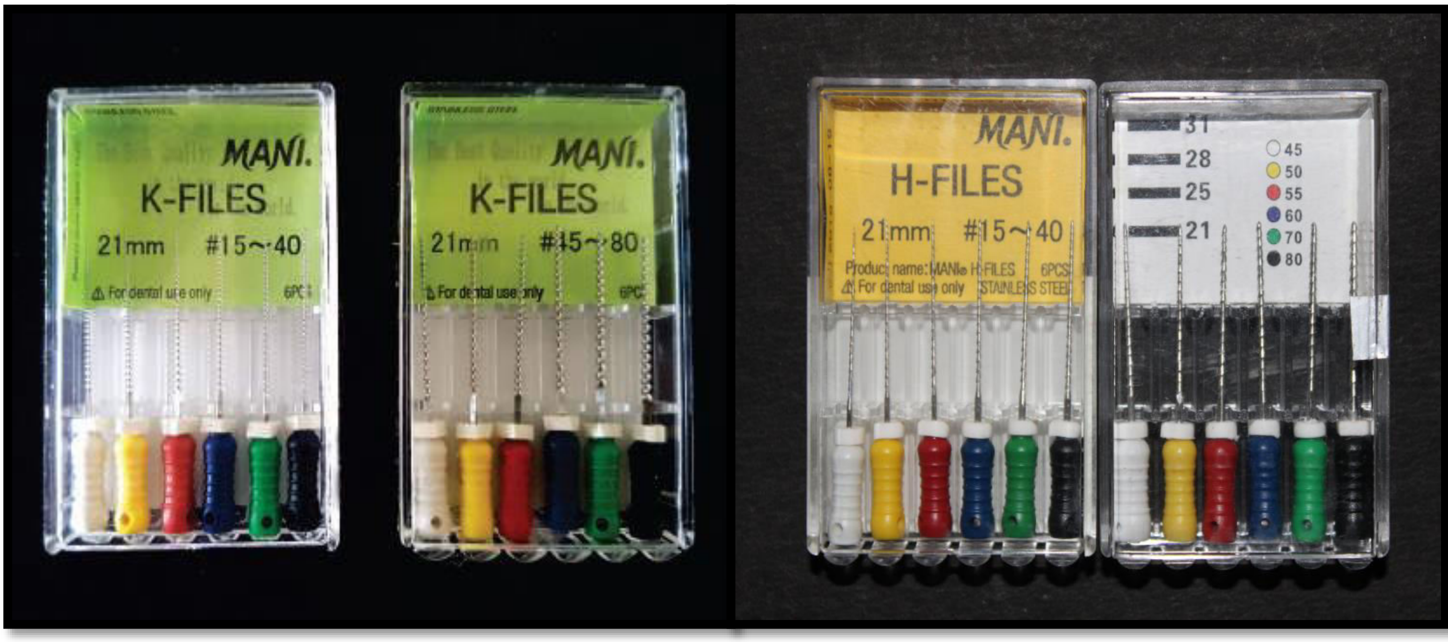

**Straight handpiece** (NSK, Japan)**, Double Sided Diamond Disc** (DSF,Germany)

PLATE -III

# ARMAMENTARIUM


**Endodontic motor X-Smart**
(DENTSPLY, Maillefer, Switzerland)
)


**Gates Glidden Drills**
(MANI, Japan)




**Standard 2% K & H Files (#15-80)** (MANI,Japan)

# PLATE -IV

## ARMAMENTARIUM


**Reamers** (MANI, Japan)

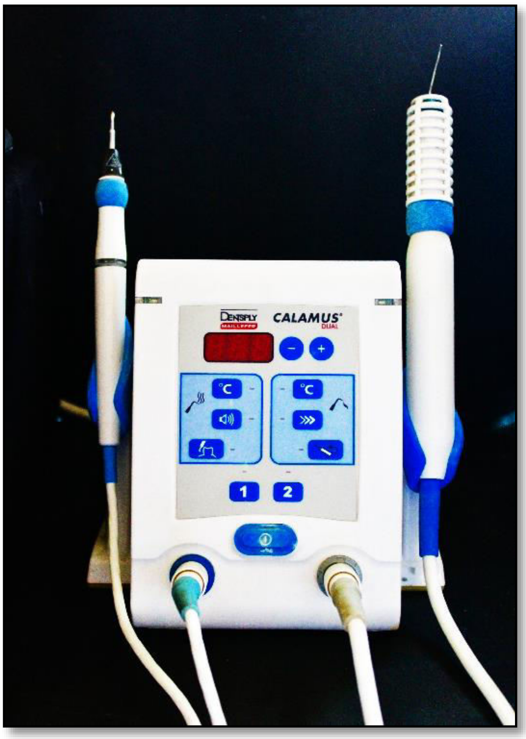
**ProTaper Universal Rotary Files**
(DENTSPLY, Maillefer, Switzerland)

**LentuloSpiral** (MANI, Japan)

PLATE -V

# ARMAMENTARIUM

**Roeko Surgi-tip Endo**
(Coltene,Switzerland)




**Calamus Dual 3D Obturation System**
( DENTSPLY, Maillefer, Switzerland)

PLATE -VI

# MATERIALS

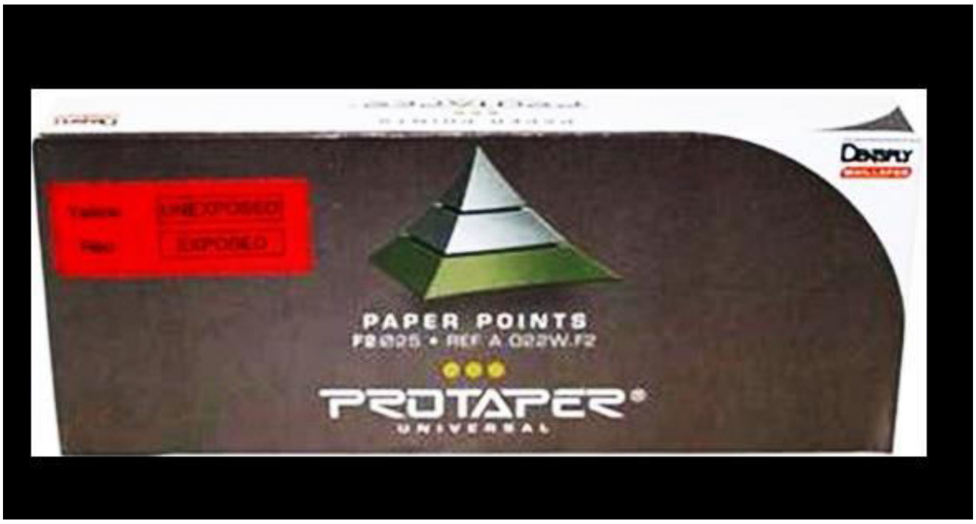

**Sodium Hypochlorite** (Hyposept UPS Hygienes)
**Normal Saline** (Nirlife)


**Irrigation Syringe , Side Venting Needle** (Nirlife)


**RC Help 17% EDTA** (Prime Dental Products)

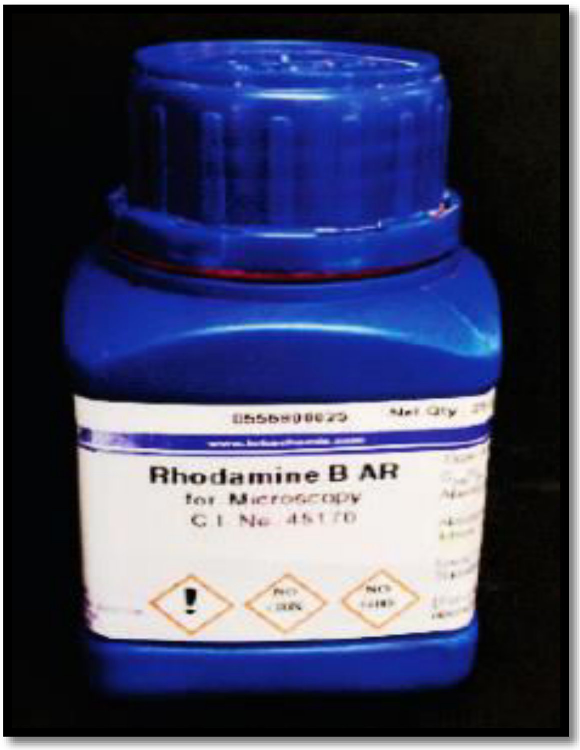
PLATE -VII

# MATERIALS




**Absorbent Paper Points**
(DENTSPLY, Maillefer, Switzerland)


**Isopropyl Alcohol**
(Cerkamed,Poland)




**Rhodamine B dye**
(Loba Chemie,India)

PLATE -VIII

# MATERIALS


**AH Plus Resin Sealer** (DENTSPLY, Maillefer, Switzerland)

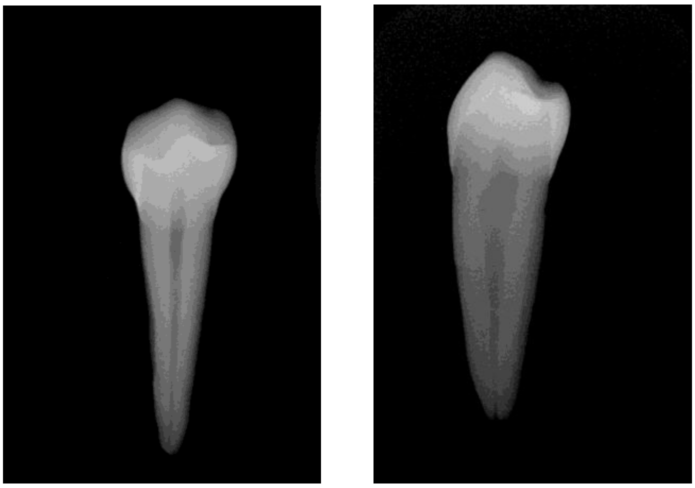

**Bioroot RCS** (SEPTODENT, France)


**Gutta Percha** points (DENTSPLY, Maillefer, Switzerland)

PLATE -IX

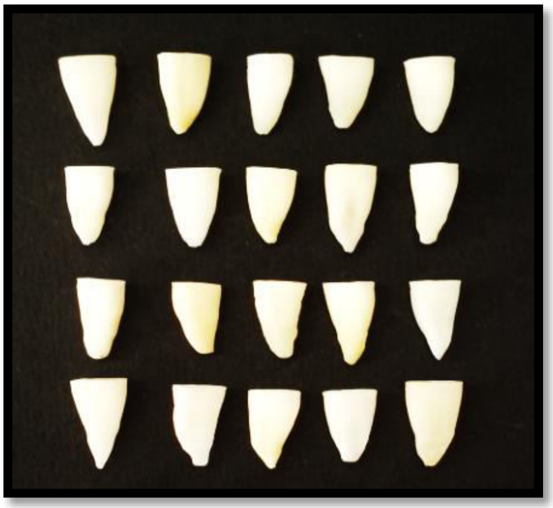
## METHODOLOGY

Sample size (N=60)



Pre Operative Radiographs

Decoronation of samples

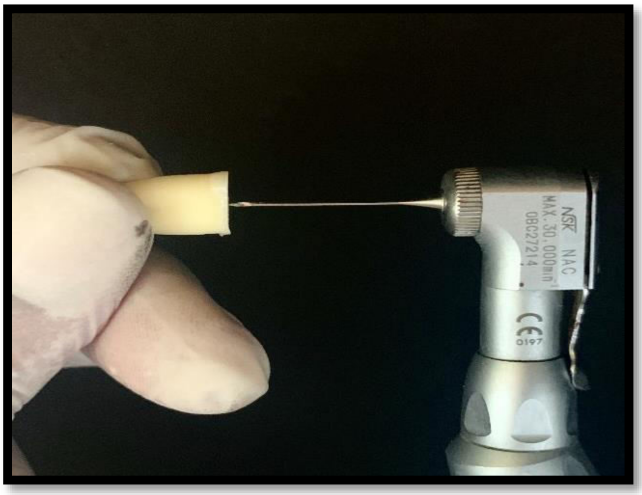


Decoronated Samples(n=20)

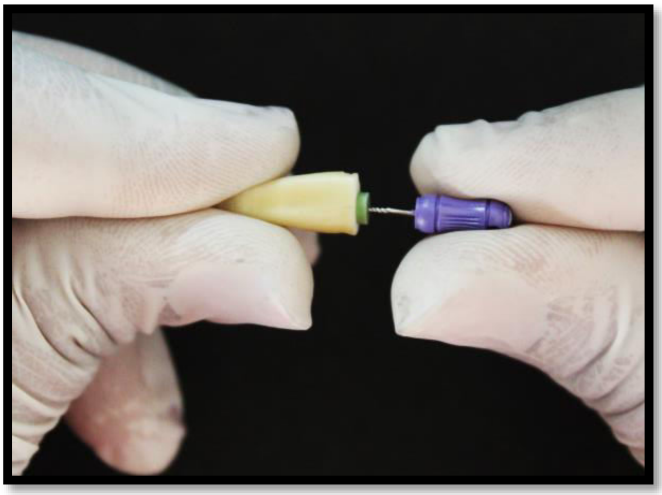

Length Measured with Digital Vernier Caliper

## PLATE -X

# METHODOLOGY

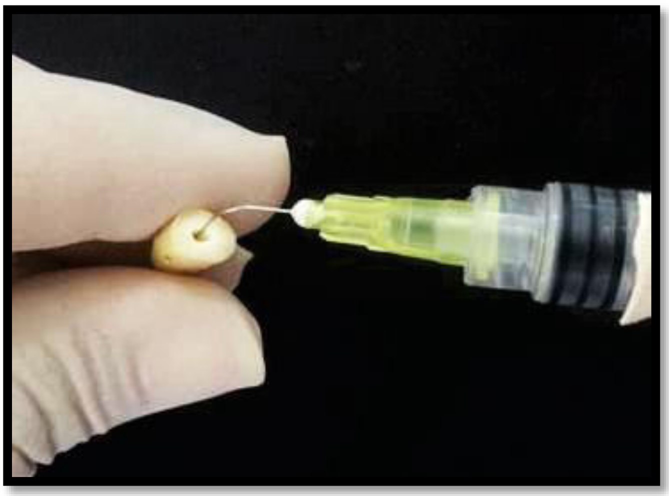


**Coronal Enlargement with Gates Glidden Drill**



**Working Length Determination**

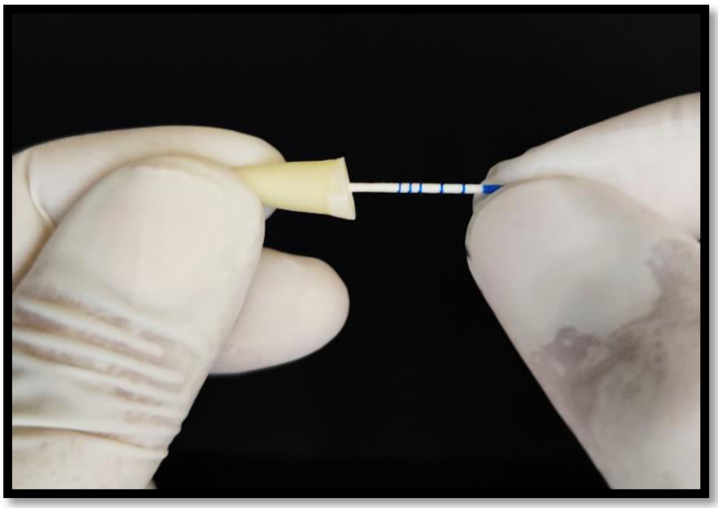
**Biomechanical preparation**



**Irrigation of Root Canal**

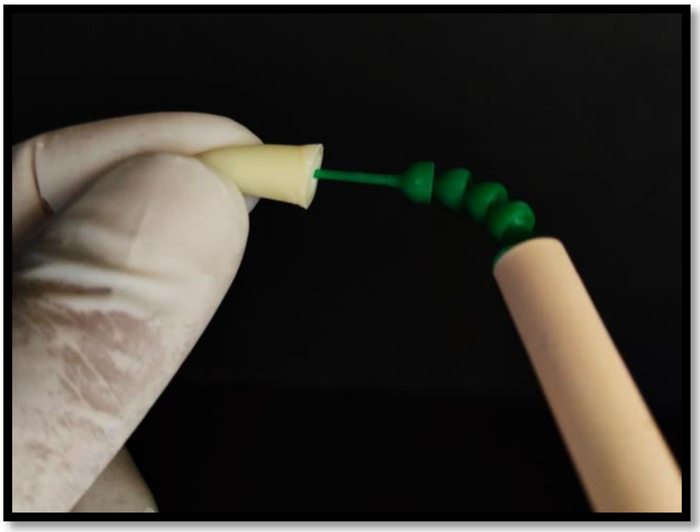
PLATE -XI

## METHODOLOGY



**Canal Drying with Paper Point**

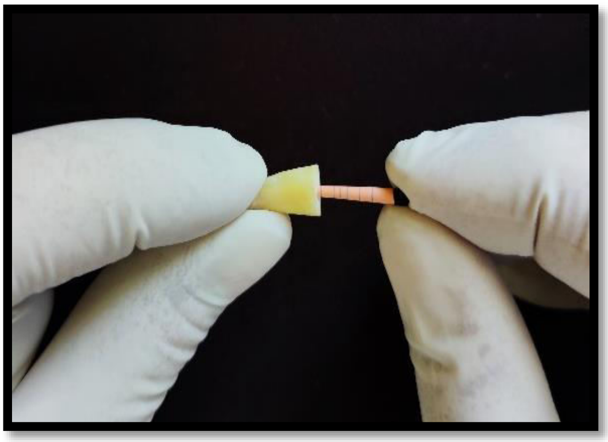

**Canal Drying with Isopropyl Alcohol**



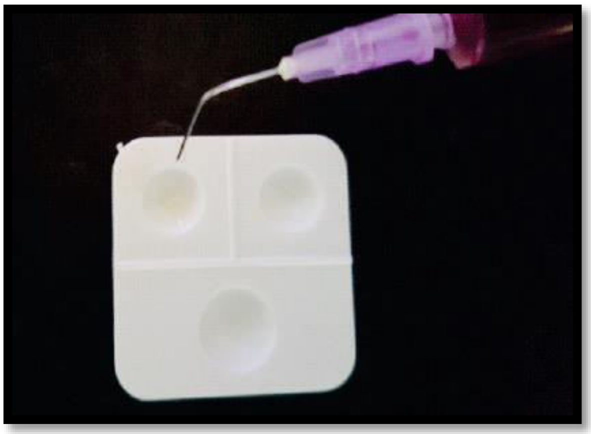
**Canal Drying with Roeko Surgitip- Endo**

# PLATE -XII

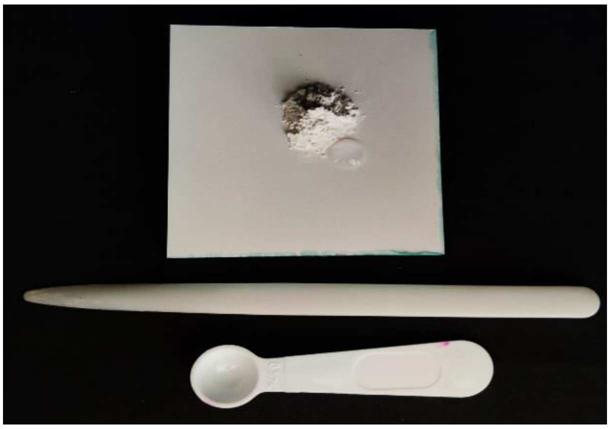
## METHODOLOGY



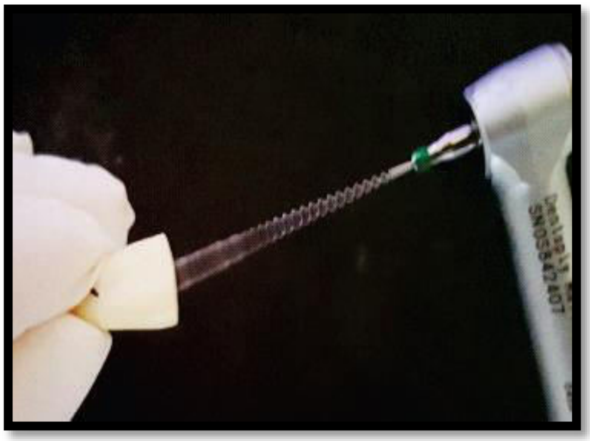
**Master Cone Selection**



**Labelling with Rhodamine B Dye**



**Manipulation of Sealer a & b**

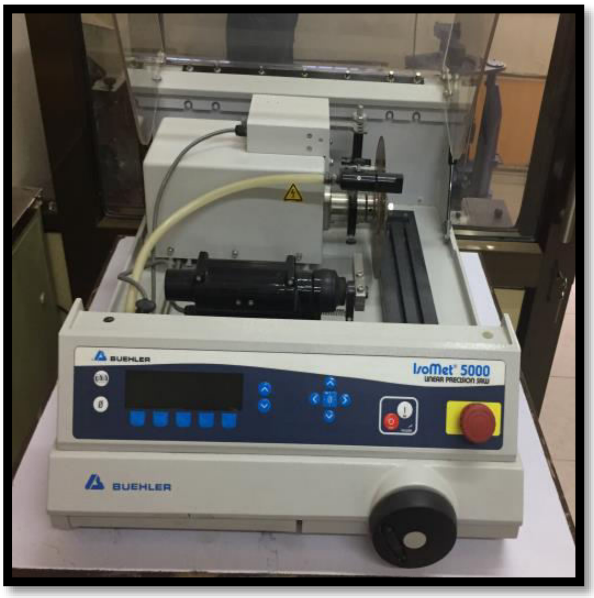


**Application of sealer with Lentulo Drill**

**Obturated Root Canal**

# PLATE -XIII

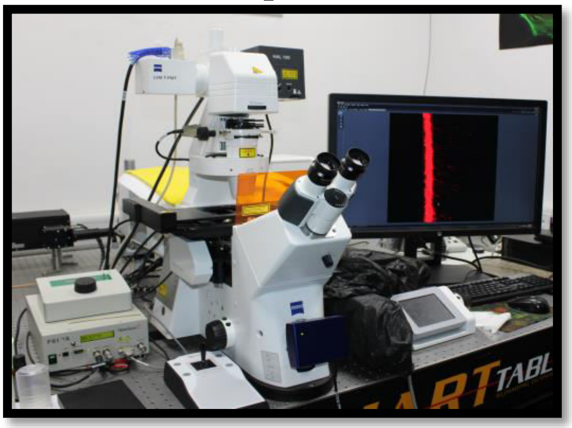
## METHODOLOGY



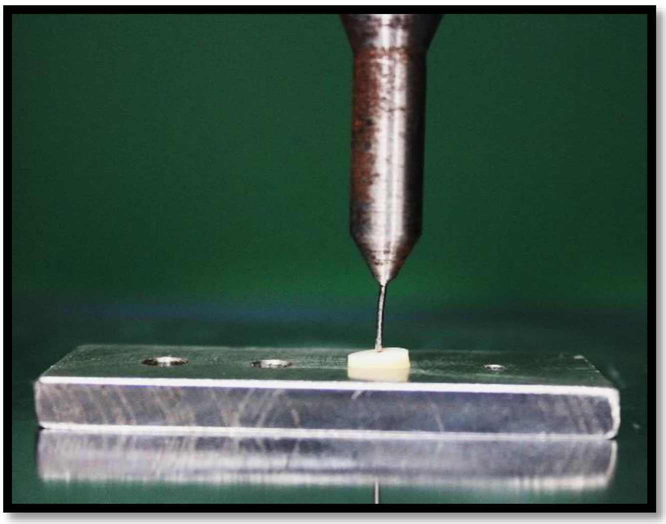

**Sectioning of samples with Precision saw** (IsoMet 5000, Buehler)

**Polishing of samples on Grinder & polisher** (Buehler)


**Sectioned Samples**



**Confocal Laser Scanning Microscope** (ZEISS with LSM Software ZEN 2007)

**Universal Testing Machine** (ACME Engineers, Model no. UNITEST-10)

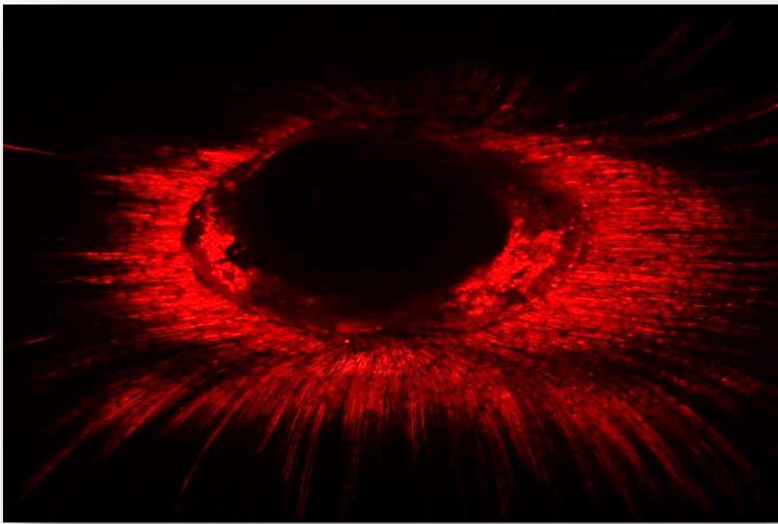
PLATE -XIV

# CONFOCAL LASER MICROSCOPIC IMAGES

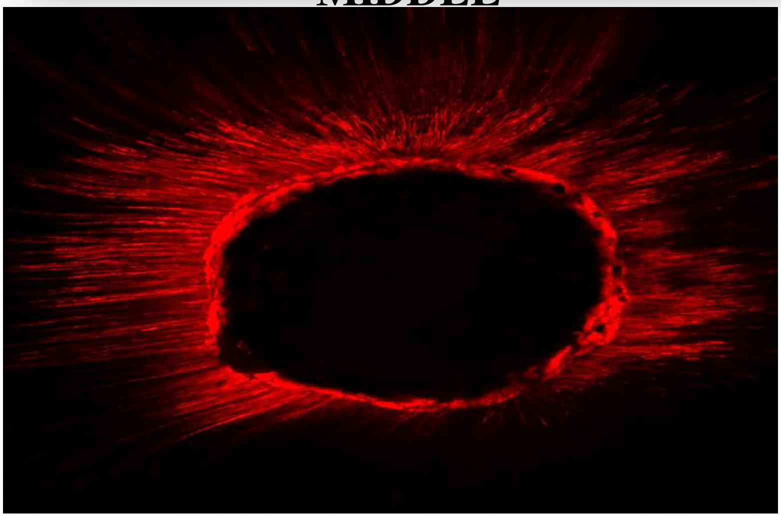
## GROUP - Ia

Paper Point with Resin based Sealer

**CORONAL**



**MIDDLE**



**APICAL**

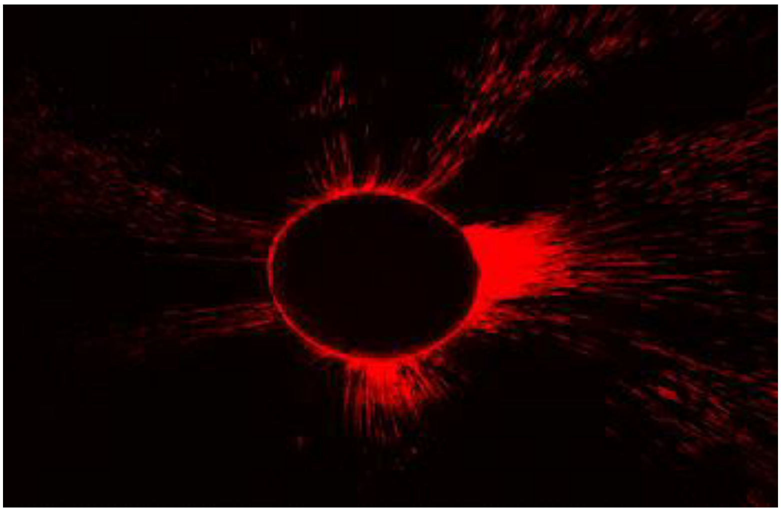


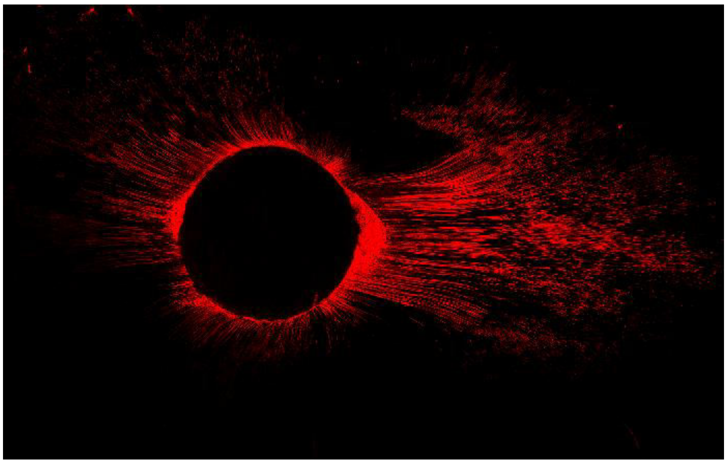
PLATE -XV

## CONFOCAL LASER MICROSCOPIC IMAGES

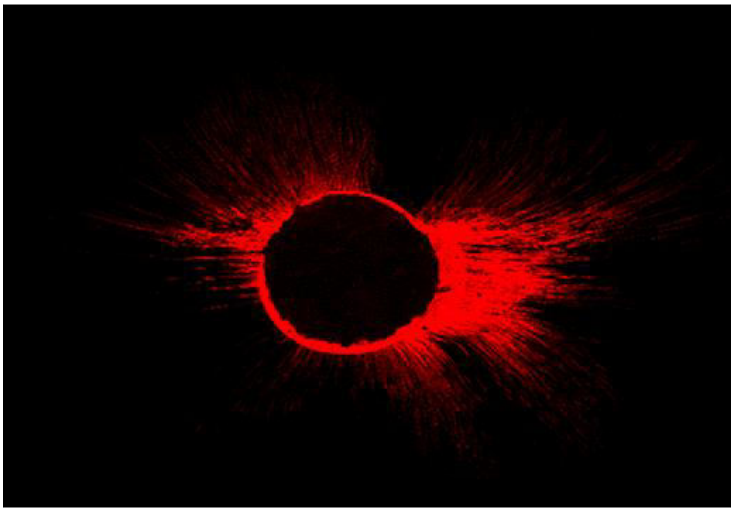
### GROUP - Ib

Paper Point with Calcium silicate based Sealer

**CORONAL**



**MIDDLE**



**APICAL**

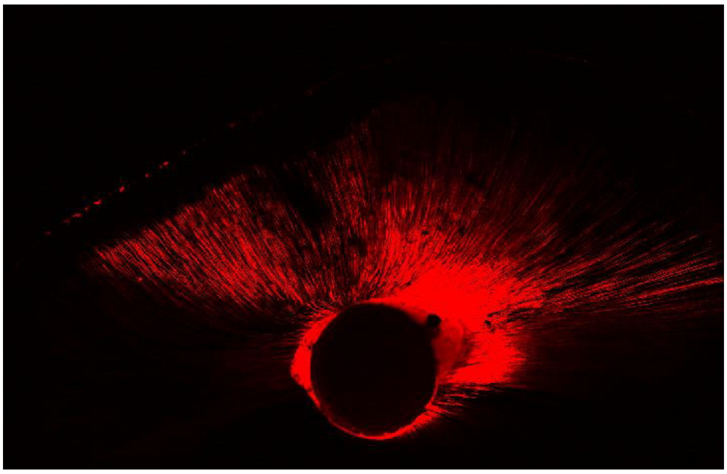


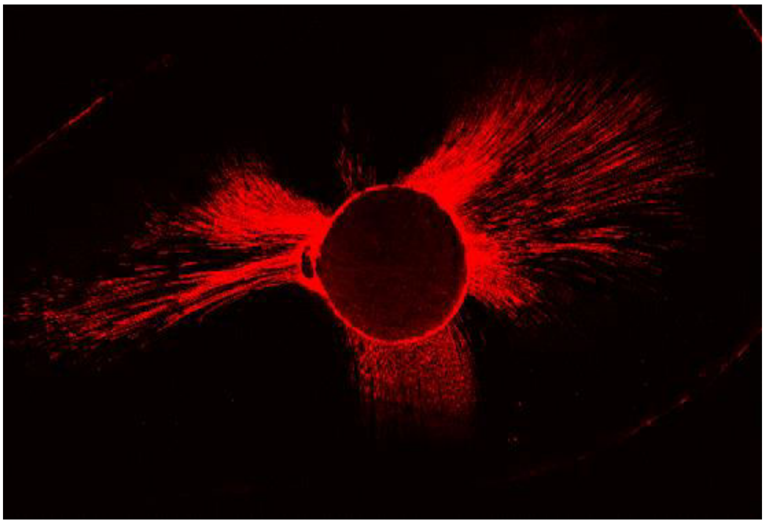
PLATE -XVI

# CONFOCAL LASER MICROSCOPIC IMAGES

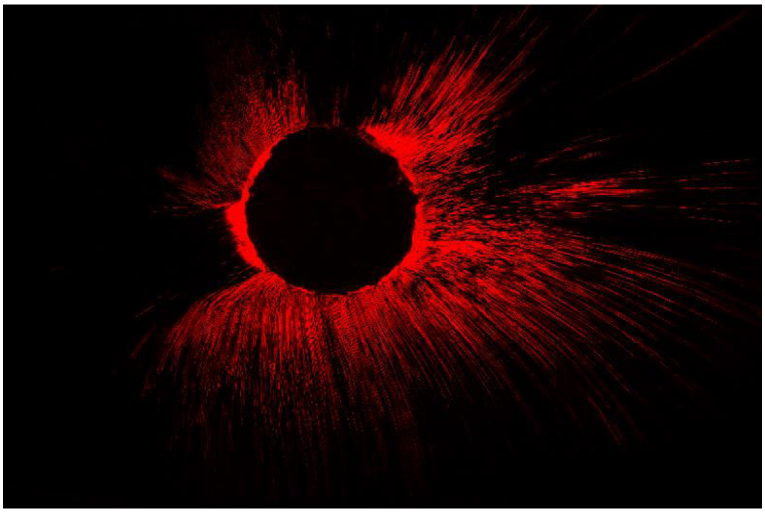
## GROUP - IIa

Isopropyl Alcohol with Resin based Sealer

**CORONAL**



**MIDDLE**



**APICAL**

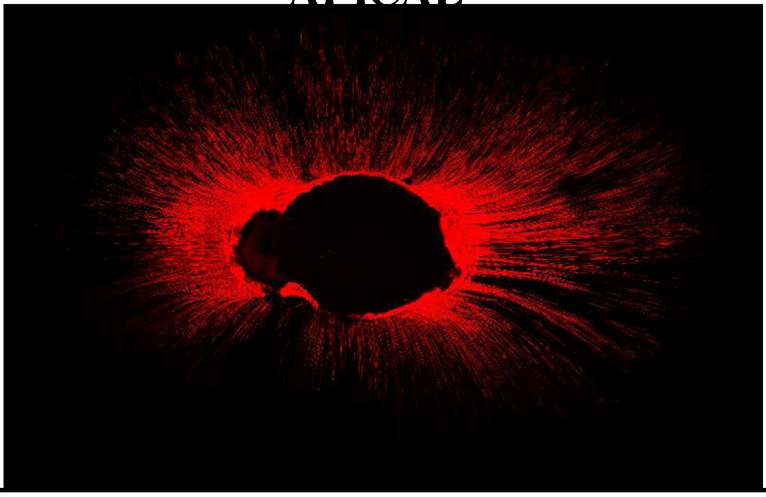


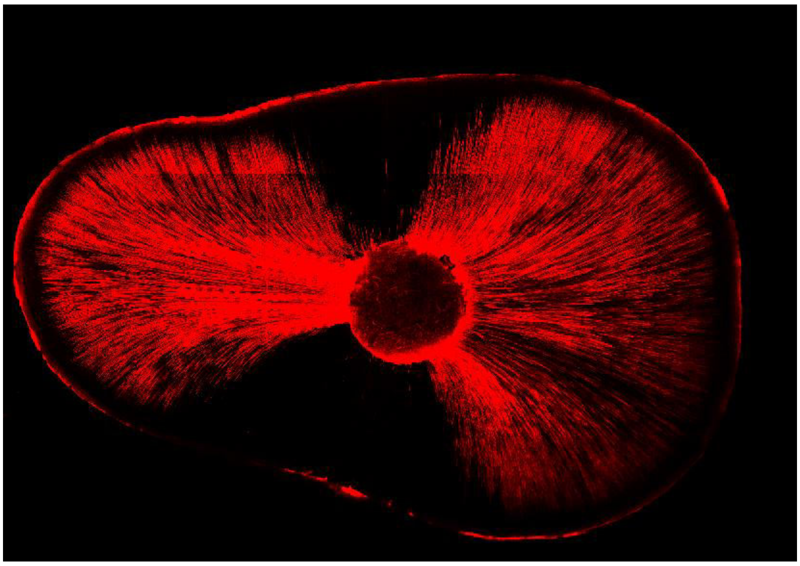
PLATE -XVII

# CONFOCAL LASER MICROSCOPIC IMAGES

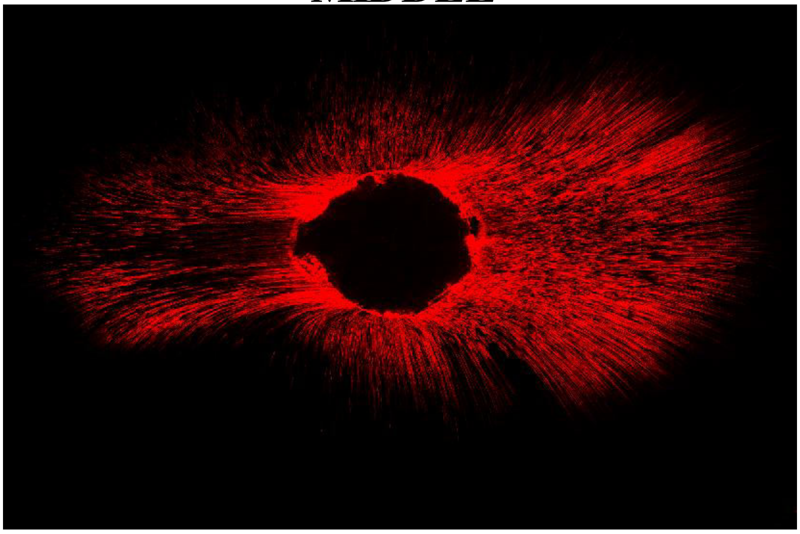
## GROUP - IIb

Isopropyl Alcohol with Calcium silicate based Sealer

**CORONAL**



**MIDDLE**



**APICAL**

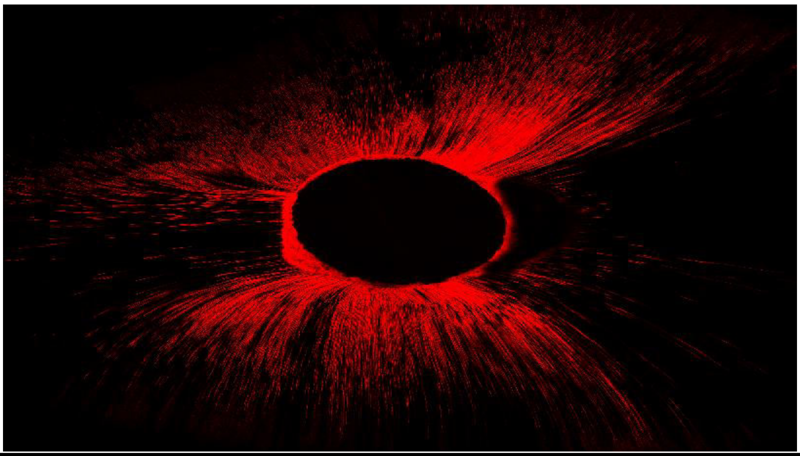


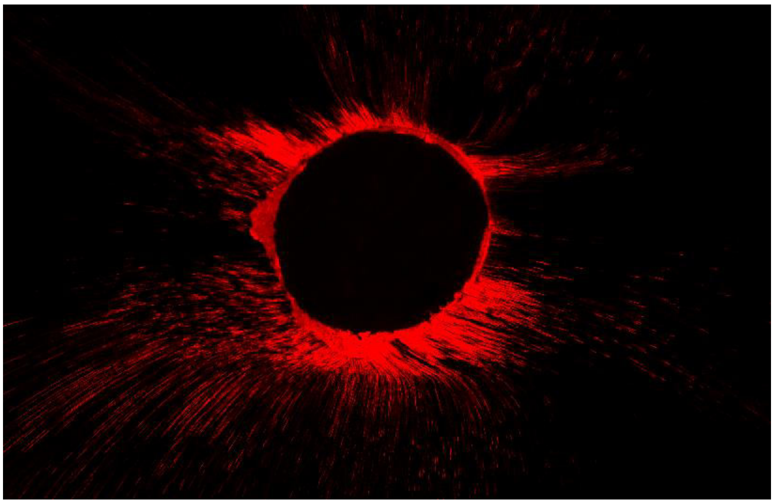
PLATE -XVIII

## CONFOCAL LASER MICROSCOPIC IMAGES

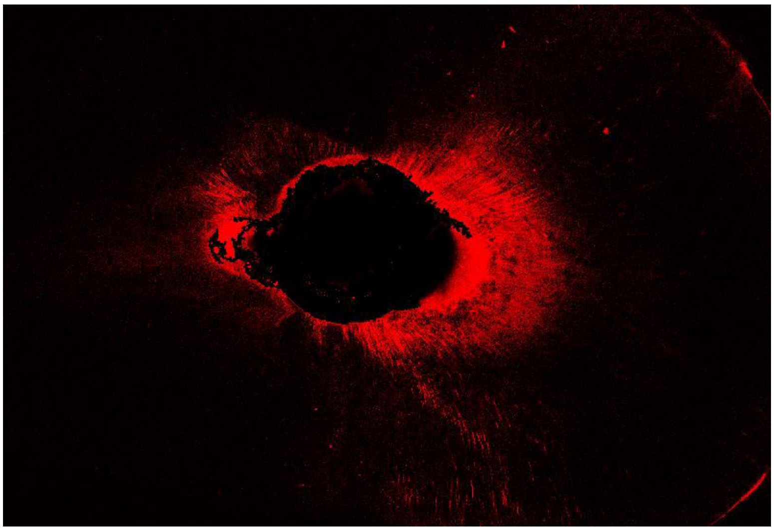
## GROUP - IIIa

Roeko surgitip Endo with Resin based Sealer

**CORONAL**



**MIDDLE**



**APICAL**

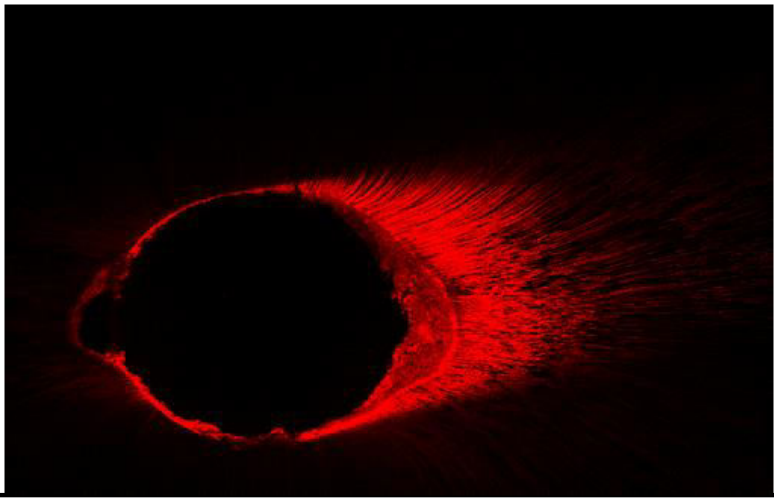


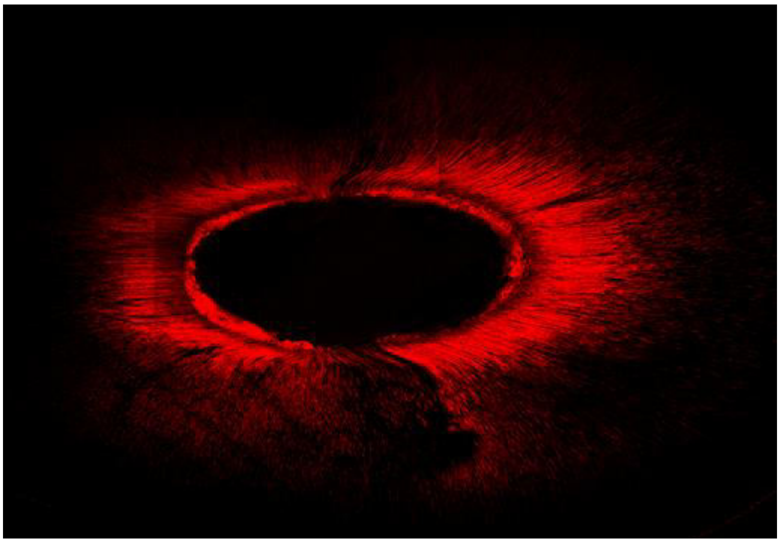
PLATE -XIX

# CONFOCAL LASER MICROSCOPIC IMAGES

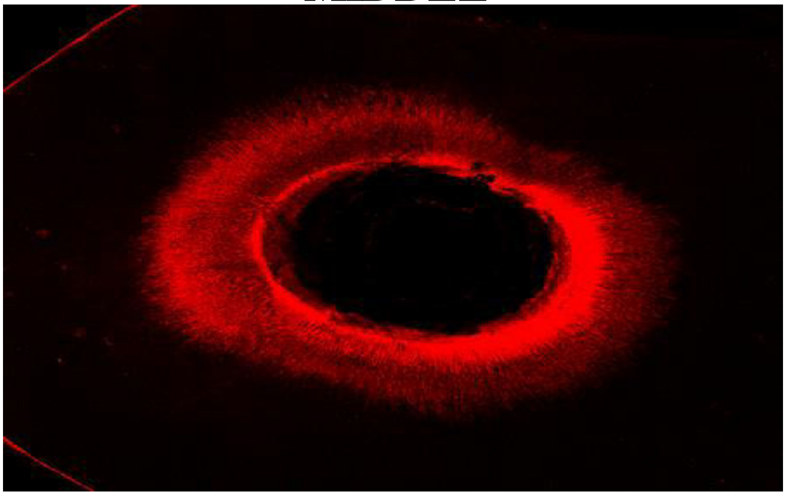
## GROUP - IIIb

Roeko surgitip Endo with Calcium silicate based Sealer

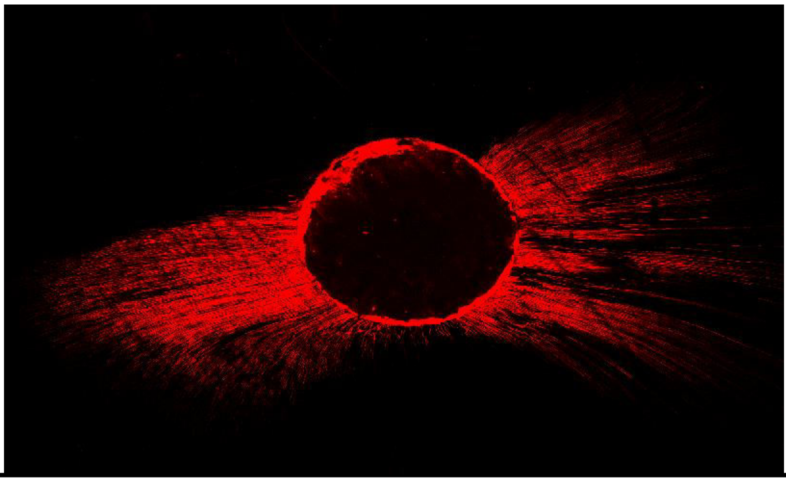
**CORONAL**



**MIDDLE**



**APICAL**



# RESULT

The present in vitro study was carried out to evaluate the effect of various drying protocol (Paper point, Isopropyl Alcohol and Roeko surgitip-Endo) on Intratubular penetration by confocal laser scanning microscope and its relation to Push out bond strength of Resin based sealer and Calcium silicate-based sealer to root dentin.

Depending upon the type of drying protocol used and type of sealer, the samples were randomly divided into six groups:

## GROUP I:- PAPER POINT

Sub Group I a:- Paper point with resin based sealer (n=10)

Sub Group I b:- Paper point with calcium silicate based sealer(n= 10)

## GROUP II: - ISOPROPYL ALCOHOl

Sub Group II a:- Isopropyl alcohol with resin based sealer( n= 10)

Sub Group II b:- Isopropyl alcohol with calcium silicate based sealer(n=10)

## GROUP III:- ROEKO SURGITIP- ENDO

Sub Group III a:- Roeko surgitip – endo with resin based sealer(n=10)

Sub Group III b:- Roeko surgitip – endo with calcium silicate based sealer(n=10)

Licensed version of SPSS 20.0 (IBM Crop) was used for statistical analysis. The data on intratubular penetration and (μm) and push out bond strength (Mpa) were obtained on samples according to three different canal drying protocols and two sealer types at coronal, middle and apical sections. The statistical summaries like mean and standard deviation were obtained for each drying protocol, each sealer type and at each section. The summaries were obtained for both intratubular penetration and push out bond strength. The comparison of mean intratubular penetration and push out bond strength between two sealer types for each canal drying protocol was performed using t-test for independent samples. Further, the comparison of these two parameters for each sealer type across canal drying protocols was performed using one-way analysis of variance (ANOVA). The paired comparison between two protocols was done using Tukey's post-hoc test. Also, the correlation between intratubular penetration and push out bond strength was determined using Pearson's correlation coefficient. The analysis was performed for each protocol and each sealer type.

All the analyses were performed using SPSS ver 20.0 (IBM CORP, USA) software and the statistical significance was tested at 5% level.

## The formulations used in the study are as below:

## 1. Measures of central tendency

If $x_1$, $x_2$,…$x_n$ are the observations on a random variable X, then following measures of central tendency can be obtained:

**Mean** for a set of observations is given by

$$\overline{x} = \frac{1}{n}\sum_{i=1}^{n} x_i$$

2. Measures of dispersion

**Standard deviation** for a set of observations is given by

$$s = \sqrt{\frac{1}{(n-1)}\sum_{i=1}^{n}(x_i - \overline{x})^2}$$

where $x_i$ = observation on each object

$n$ = number of objects

3. Statistical inference tests

## Student's t-test for independent samples

The test is used for comparing the statistical significance of difference in the means of two samples. It compares the sample difference between two means in relation to the variation in the data (expressed as the standard deviation of the difference between the means).

**It is given by the formula:**

$$t = \frac{(\bar{x}_1 - \bar{x}_2) - (\mu_1 - \mu_2)}{S\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

where $\bar{x}_1$ and $\bar{x}_2$ are the means of sample observations of two different groups, $\mu_1$ and $\mu_2$ are the means of the respective populations from which the samples are derived, and *S* is the pooled sample standard deviation, which is given by:

$$s^2{}_{pooled} = \frac{(n_1 - 1)s_1{}^2 + (n_2 - 1)s_2{}^2}{n_1 + n_2 - 2}$$

here $s_1{}^2$ and $s_2{}^2$ are the variance of two samples and $n_1$ and $n_2$ are the sample sizes in two groups. If the test statistic results in a *P*-value > 0.05 (level of significance), then the null hypothesis $H_0$: *There is insignificant difference in the means of two groups* is accepted and the alternative hypothesis $H_1$: *There is significant difference in the means* is rejected. On the other hand, if *P*- value < 0.05, then the $H_1$ is accepted and $H_0$ is rejected.

## One-way Analysis of variance

Analysis of variance (ANOVA) is used to test the significance of difference in the $H_0 : \mu_1 = \mu_2 = ...\mu_m$ mean of three or more groups. The basic assumption is that the variable of interest is normally distributed in the population under study.

## Method

Here the interest is to test the null hypothesis that the population means are same, i.e.

against the alternative $H_1$ that they are not same.

Some of the statistics computed to test the hypothesis are as below:

## Some of the statistics computed to test the hypothesis are as below:

**i)** **Grand mean**: It is the mean of set of all observations in the studied groups and is given by:

$$\overline{x}_{GM} = \frac{1}{N}\sum_{i=1}^{N} x_i$$

**ii)** **Total sum of squares:** It is the sum of squares of each observation from the grand mean and is given by:

$$TSS = \sum_{i=1}^{N}(x_i - \overline{x}_{GM})^2$$

Total sums of squares is the sum of two components i.e., variation between groups and within groups.

### iii) Between group sum of squares

$$SSB = \sum_{j=1}^{m} n_j(\overline{x}_j - \overline{x}_{GM})^2$$

## iv) Within group sum of squares

$$SSW = \sum_{j=1}^{m}\sum_{i=1}^{n}(x_{ij} - \overline{x}_j)^2$$

The mean sum of squares is obtained by dividing the above sum of squares with the respective degrees of freedom, i.e. $N$-1, $p$-1 and $p(n$-1).

## v) F-statistic:

It is the ratio of between and within mean sum of squares

$$F = \frac{MS_{Between}}{MS_{Within}}$$

If the $p$-value based on F-statistic is greater than 0.05, $H_0$ is accepted, otherwise $H_1$ is accepted.

## vi) Tukey's post-hoc test

After performing ANOVA, if alternative hypothesis $H_1$ is accepted, then the subsequent interest is to determine the pair wise significance of difference in the means of study groups. This could be carried using Tukey's post-hoc test. The difference between the means of all groups are determined and compared with this critical difference called the honest significant difference (HSD). It is given by:

$$HSD = q\sqrt{\frac{MS_{within}}{n}}$$

where, *q* is the studentized range statistic derived from the tables, *n* is the sample size and the mean square value is from the ANOVA analysis. If the critical difference exceeds the absolute difference between any two sample means, then the corresponding means differ significantly.

## Pearson's correlation

Pearson's correlation coefficient quantifies the relationship between two measurable variables. It measures the linear relationship between two variables. Thus, if *X* and *Y* are two variables taking values $x_1$, $x_2$,…$x_n$ and $y_1$, $y_2$,…$y_n$, then the correlation coefficient (*r*) between the two variables is given by:

$$r = \frac{n(\Sigma xy) - (\Sigma x)(\Sigma y)}{\sqrt{[n\Sigma x^2 - (\Sigma x)^2][n\Sigma y^2 - (\Sigma y)^2]}}$$

The value of *r* lies between -1 to +1, with -1 indicating perfect negative correlation and +1 indicating perfect positive correlation.

## Overall Results

The mean values and standard deviations of intratubular penetration and push-out bond strength for three different drying protocols namely Paper Point (Group I), Isopropyl Alcohol (group II), Roeko surgitip Endo (Group III) and Sealers (Resin Based Sealer and Calcium silicate-based sealer) at Coronal, Middle and Apical sections have been described in Table.1and Table.3 respectively.

The maximum intratubular penetration (1080.94) was observed in Group

IIb at coronal level whereas the minimum intratubular penetration (100.70) was observed in Group IIIa at apical level. The maximum push-out bond strength (4.53) was observed in Group IIb at coronal level whereas the minimum pushout bond strength (0.60) was observed in Group IIIa at apical level.

Higher values for intratubular penetration and push-out bond strength were obtained for group in which Isopropyl alcohol was used as compared to those in which Paper point and Roeko surgitip endo was used as drying protocol. Higher values for intratubular penetration and push-out bond strength were obtained for groups in which Bio Root sealer was used as compared to those where AH Plus sealer were used, irrespective of the drying protocol used.

Higher values for intratubular penetration and push-out bond strength were also obtained at coronal section as compared to middle and apical sections irrespective of drying protocol used for drying the root canals. (Table.1 and Table.2a, 2b, 2c, Figure 1and Figure 2).

## Analysis for Intratubular Penetration:

In order to determine the effect of various drying protocol on intratubular penetration of sealers, two-way analysis of variance was performed with groups as fixed effects. This analysis was performed at coronal, middle and apical sections, independently. (Table1, Figure 1)

At coronal, the difference of mean intratubular penetration for Resin based sealer, across three drying protocols was statistically significant with a p-value of $< 0.0001$, using one-way analysis of variance. Pair wise comparisons using Tukey's post-hoc test revealed that the differences between Paper point

(Group Ia) and Roeko surgitip-endo (Group IIIa) as well as Isopropyl alcohol (Group IIa) and Roeko surgitip-endo were statistically significant with p-values 0.001 and < 0.0001 respectively.

Similarly, the comparison of mean penetration for Calcium silicate was performed across three protocols. At coronal section, the difference in the means was statistically significant with a p-value of 0.002. Pair wise comparisons using Tukey's post-hoc test revealed that the differences between Paper point (Group Ib) and Roeko surgitip-endo (Group IIIb) as well as Isopropyl alcohol (Group IIb) and Roeko surgitip-endo (Group IIIb) were statistically significant with p-values 0.015 and 0.002 respectively. (Table 1, Table 2a, Table 2b, Table 2c, Figure 1, figure 2)

In the middle section, the difference of mean penetration for Resin based sealer across three protocols was statistically significant with a p-value of 0.005. Paired comparisons revealed that the difference of means between Isopropyl alcohol (Group IIa) and Roeko surgitip-endo (Group IIIa) protocols was significant with a p-value of 0.004. Similarly, the comparison of means for Calcium silicate across three protocols at middle section revealed statistically significant difference with a p-value of 0.003. The paired comparison showed significant difference of means between Isopropyl alcohol (Group IIb) and Roeko surgitip-endo (Group IIIb) protocols with a p-value of 0.002. (Table 1, Table 2a, Table 2b, Table 2c, Figure 1, figure 2)

In the apical section, the difference in the means was statistically insignificant across protocols for both the sealer types. (Table 1, Table 2a, Table 2b, Table 2c, Figure 1, figure 2)

## Analysis for Push-Out Bond Strength:

In order to determine the effect of various drying protocol on push-out bond strength, two-way analysis of variance was performed with groups as fixed effects. This analysis was performed at coronal, middle and apical sections, independently.

At coronal section, the difference in the means for Resin based sealer across protocols was statistically significant with a p-value of 0.001, using one-way analysis of variance. Pair wise comparisons using Tukey's post-hoc test revealed that the differences between Paper point (Group Ia) and Roeko surgitip-endo (Group IIIa) as well as Isopropyl alcohol (Group IIa) and Roeko surgitip-endo (Group IIIa) were statistically significant with p-values 0.029 and 0.001 respectively. On similar lines, the comparison of means was performed for Calcium silicate-based sealer. The difference in the means was statistically significant with a p-value of < 0.0001. Pair wise comparisons using Tukey's post-hoc test revealed that the differences between Paper point (Group Ib) and Roeko surgitip-endo (Group IIIb) as well as Isopropyl alcohol (Group IIb) and Roeko surgitip-endo (Group IIIb) were statistically significant with p-values 0.031 and < 0.0001 respectively. (Table 3, Table 4a, Table 4b, Table 4c, Figure 3, figure 4)

At middle section the difference in the means for Resin based sealer across protocols was statistically significant with a p-value of 0.045. Paired comparisons revealed that the difference of means between Isopropyl alcohol (Group IIa) and Roeko surgitip-endo (Group IIIa) protocols was significant with p-values 0.049. The analysis for Calcium silicate-based sealer revealed that the difference in the means was statistically significant with a p-value of < 0.0001. Paired comparisons revealed that the difference of means between Paper point (Group Ib) and Roeko surgitip-endo (Group IIIb) as well as Isopropyl alcohol (Group IIb) and Roeko surgitip-endo(Group IIIb) protocols were significant with p-values 0.011 and < 0.0001 respectively. (Table 3, Table 4a, Table 4b, Table 4c, Figure 3, figure 4)

At apical section, the difference in the means for Resin based sealer was statistically significant across protocols with a p-value < 0.0001. Paired comparisons revealed that the difference between Paper point (Group Ia) and Roeko surgitip-endo (Group IIIa) as well as Isopropyl alcohol (Group IIa) and Roeko surgitip-endo (Group IIIa) were significant with p-values < 0.0001. Further, the analysis for Calcium silicate based sealer revealed that the difference in the means was statistically significant across protocols with a p-value < 0.0001. All the paired differences showed statistical significance with a p-value < 0.0001. (Table 3, Table 4a, Table 4b, Table 4c, Figure 3, figure 4)

## Analysis of correlation between intratubular penetration and pushout bond strength:

In order to determine the Correlation between intratubular penetration and pushout bond strength for each combination of canal drying protocol and sealer type, two-way analysis of variance was performed with groups as fixed effects. This analysis was performed for paper point, Isopropyl Alcohol and Roeko Surgitip- endo independently. (Table 5, Figure 5)

For Paper point protocol and Resin based sealer, the correlation was low positive (0.363), and was statistically significant with a p-value of 0.047, while for Calcium silicate sealer, the correlation between two parameters was moderately positive (0.380) and was statistically significant with a p-value 0.037.

For Isopropyl alcohol protocol and Resin based sealer, the correlation was low positive (0.365) and was statistically significant with a p-value 0.047, while for Calcium silicate sealer, the correlation between the parameters was low positive (0.403) and was statistically significant with a p-value 0.027.

For Roeko surgitip-endo protocol and Resin based sealer, the correlation was moderately positive (0.348) and was statistically insignificant with a p-value 0.059. For Calcium silicate sealer, the correlation between the parameters was low positive (0.360) and was statistically significant with a p-value 0.048.

# DISCUSSION

Three dimensional impervious obturation of the root canal system is of prime clinical importance for the long-term success of endodontic treatment.[50]

The main components of an obturation are: a solid or semisolid core such as gutta-percha and a sealer. Gutta-percha which occupies bulk of the canal space, has no adhesive qualities to dentin regardless of the obturation techniques used.[51] Therefore, root canal sealers play a major role in achieving the fluid tight seal by filling the interface between the core material and the dentin wall, accessory and lateral canals, voids, spaces and irregularities between gutta-percha. The choice of sealer is not only dependent on its ability to create a sound seal, but it must also be well tolerated by the peri radicular tissues and be relatively easy to manipulate so that its optimum physical properties can be achieved.[52]

Traditionally, Zinc Oxide Eugenol was the most commonly used sealer.

However, there are several disadvantages associated with this conventional sealer such as: non adhesive, irritant to periapical tissue and carcinogenic.

Later, introduction of epoxy resin based and calcium silicate-based sealers brought a revolution in the field of dentistry by providing a reliable substitute for conventional sealers.

Epoxy resin-based sealers were introduced in endodontics by **Schroeder et al.**[53]

**Harpreet singh et al. (2014)**[54] stated that these sealers possess very good physical properties, excellent apical sealing and ensure adequate biological performance.

Moisture condition of root canals affect quality of adhesion between root canal dentin and sealers before filling procedures [16,17] **Zemner et al. (2008)** stated that different levels of residual moisture in the root canal have shown to alter the sealing properties of conventional and resin-based sealers.[23] Various drying protocols have been recommended to maintain root canals in a moist state and to improve the dentin hybridization of hydrophilic sealers.

Previous researchers have shown that intratubular penetration and bond strength of sealers to root canals may be influenced by various drying protocols used prior to obturation. Thus, achieving reliable bonding and effective adhesion inside the root canal is still an issue of interest. There is scarcity of research on the effect of various drying protocols on sealers intratubular penetration and its relation with push-out bond strength. Hence, the following study was carried out to evaluate effect of various drying protocol (Paper point, Isopropyl Alcohol and Roeko surgitip-Endo) on intratubular penetration and push out bond strength of Resin based sealer and Calcium silicate-based sealer to root dentin using Confocal Laser Scanning Microscopy (CLSM) and its relation to push-out bond strength.

Sixty freshly extracted human mandibular first premolars were selected for the study. The teeth were collected and stored in phosphate-buffered saline for not more than 12 weeks as suggested by **Jameson MW et al. (1994).**[49] He had observed that storage media and time of the specimen storage affect the tooth after extraction due to water loss with dehydration of dentin. Phosphate-buffered saline shows the best compatibility in maintaining the hydration of the extracted teeth.[49]

For sample size estimation, a study by **Dias KC et al. (2014)**[20] was referred. Higher bond-strength was seen in Isopropyl alcohol group along with resin based sealer (4.71 ± 1.0) at coronal level and least was seen in Paper Point group (0.22 ± 0.08) at apical level. Assuming that similar differences could be obtained in the present study, the estimated sample size that could provide 80% power and 95% confidence interval was 10 samples per group. Therefore, the total sample size for the current research was kept 60.

## The formulation used was:

$$n = \frac{(z_{1-\alpha/2\tau} + z_{1-\beta})^2}{ES^2}$$

where $\tau$ is the number of possible comparisons, $z_{1-\alpha/2\tau}$ (2.409) is the standardized value for 5% error and for 3 paired comparisons, $z_{1-\beta}$ (1.282) is the value for 90% power and ES is the effect size (*f*).

Mandibular First Premolars are the most commonly extracted teeth for orthodontic extraction. They also present a unique combination of occlusal dynamics, structural loading and anatomical design. They have a bulkier crown and yet are supported with more slender and shorter root. Also, they are more likely subjected to lateral forces during mastication.[55] All these factors predispose the tooth to traumatic dental injuries. Hence in the current study, mandibular first premolars were selected as samples.

Decoronation of sample was done at predetermined distance i.e. 15±1 mm from apex using water cooled diamond disc perpendicular to long axis of tooth and the cut coronal surface was ground flat using an abrasive paper. This led to standardization and uniformity in samples.[56]

One of the methods of measuring the working length on extracted teeth is to insert an endodontic file into the root canal until the tip of the file was just visible at the apical foramen. The stopper was adjusted to the reference point and the file was withdrawn. The canal length was determined and the working length was established by deducting 1 mm from this length; these readings were registered as actual working length (AWL). This method as described by **Shanmugaraj M et al.** was employed in the study.[57]

Success of endodontic treatment depends on strict adherence to "Endodontic triad". Biomechanical preparation is recognized as one of the most important step in root canal treatment**. Dafalla A et al. (2010)**[58] found that NiTi rotary files prepare canals more rapidly, and shows low incidences of blockage, and only limited loss of working length. Canal preparation with K-file was time consuming and showed higher incidence of deformed instruments probably due to low elasticity of the stainless-steel metal. **Ataide I et al.**[59] concluded that Nickel-titanium rotary instruments demonstrate a superior quality of canal preparation compared to stainless steel K files, with respect to canal cleanliness, canal transportation and canal shape. Pro-Taper Universal rotary instruments demonstrated a comparatively better quality of canal preparation in the apical region of teeth. Therefore, in the present study, the root canals were prepared by Pro-Taper Universal rotary instruments till F4 size (40/.06) under constant irrigation with 5.25% NaOCl using a 30-gauge needle.

Irrigation regimen described by **Zehnder et al. 2006**[60] was employed in the study. Between each instrument, canals were irrigated using copious amounts of the 5.25% Sodium hypochlorite solution. Once the shaping procedure was completed, canals were thoroughly rinsed using aqueous 17% EDTA followed by final rinse with distilled water.

In the present study, after irrigation protocol, block randomization of samples was done into three groups and three subgroups corresponding to treatment with different drying protocols and sealers used respectively.

## GROUP I:- PAPER POINT

Sub Group I a:- Paper point with resin based sealer (n=10)

Sub Group I b:- Paper point with calcium silicate based sealer(n= 10)

## GROUP II: - ISOPRPYL ALCOHOl

Sub Group II a:- Isopropyl alcohol with resin based sealer( n= 10)

Sub Group II b:- Isopropyl alcohol with calcium silicate based sealer(n=10)

## GROUP III:- ROEKO SURGITI- ENDO

Sub Group III a :- Roeko surgi tip – endo with resin based sealer(n=10)

Sub Group III b:- Roeko surgitip – endo with calcium silicate based sealer(n=10)

Root canal sealers are used in combination with gutta percha for an endodontic obturation. **Borges H A et al. (2014)**[61] studied physicochemical property of AH plus as compared to other conventional sealers and concluded AH Plus as gold standard. Although resin-based sealers have good properties, some researchers have shown increasing interest in materials capable of enhancing periapical tissue repair.

**Siboni et al. (2017)**[62] reported BioRoot Root canal sealer had better bioactivity with calcium release, strong alkalizing activity and apatite-forming ability, and adequate radiopacity when compared to other sealers. BioRoot RCS has gained in popularity, as it shows fewer toxic effects on human periodontal ligament cells and induces osteogenic growth factor secretion. **Alsubait et al. (2018)**[63] reported cells in the BioRoot RCS extract spread better than those in the AH Plus extract, and its particles create mineral plugs through interactions with dentinal fluids. So, In present study Bio Root root canal sealer was used in comparison with AH Plus sealer to evaluate the effect of various drying protocol on sealers intratubular penetration and its relation to push out bond strength.

In present study, 0.1% Rhodamine B dye was mixed with equal drops of AH Plus and BioRoot Sealer. Mixing of both the sealer was done according to manufacturer's manual. **Monticelli F et al. (2006)**[64] emphasized that it is very important to follow the manufacturer's recommendation to achieve an optimum bonding to root canal dentin with any type of dentin adhesives.

**Dash K A et al. (2017)**[65] reported that the depth and percentage of sealer penetration are influenced by the type of placement technique and by the root canal level, with penetration decreasing apically. Lentulo spiral has shown better penetration of sealer than the bidirectional file and ultrasonics. In present study, Sealer was introduced into the canal orifice with a Lentulo spiral (Dentsply Maillefer, Switzerland) rotated at 500 rpm in a clockwise direction with a slow-speed handpiece inserted up to 1 mm short of the WL.

**Mohan Kumar et al. (2015)**[66] stated that thermoplasticized gutta-percha technique had better adaptability to the canal walls when compared to the flowable gutta-percha (GuttaFlow) obturation and lateral condensation techniques. Thus, root canal was obturated by means of thermoplasticized obturation technique in the present study.

In the current study, after obturation, samples were sectioned at 1,4 and 7 mm from CEJ. Each section represented the coronal, middle and apical part of the obturation. As studies on the morphology of root canal dentin showed that number of tubules decreases from the crown to the apex, the response to irrigation and consequently dentine bonding can vary among different areas of the same root canal. [67,68] The samples were kept in a humidifier.

Several microscopy techniques are currently used to evaluate the intratubular penetration of sealers, including stereomicroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and confocal laser scanning microscopy (CLSM). In comparison to conventional SEM, CLSM has the advantage of providing detailed information about the presence and distribution of dental adhesives inside dentinal tubules in the total circumference of the root canal walls at relative low magnification as 100× than that of SEM because of its non-destructive nature. Also, CLSM offers improved rejection of out-of-focus noise and provides greater resolution than conventional imaging, yielding greatly enhanced images of biological structures. Further, drying of samples is not necessary with CLSM, which is indispensable for conventional microscopic technique which leads to decrease risk of shrinking or other drying artefacts. An additional feature of the confocal principal is that it permits visualization of not only a specimen surface, but also its subsurface. Thus, in the current research CLSM was preferred over SEM and TEM and the sections were observed under the confocal laser scanning microscope.[69]

For determining the bond strength of sealer to root dentin, push-out bond strength test has been considered is a reliable technique to measure the bond strength of root canal filling materials to root dentin.

Thus, the push out bond strength approach was adopted in the present study and the same specimens were subjected to axial compression force until fracture under universal testing machine at the cross-head speed of 1mm/min. This speed has used by various authors in previous studies to measure the fracture resistance of teeth using the UTM. [20]

To reduce the variability, all the samples were prepared and investigated by one operator using the standard technique.

## I) Effect of different drying protocols on intratubular penetration:

Dentinal tubule penetration depth is considered as performance measure of a root canal sealer. Previous studies have shown that the penetration of sealer into the dentinal tubules forms a physical barrier and entombs residual bacteria and improves retention of the root filling.[70,71] **Goracci C et al. (2004)** showed that the penetration of root canal sealers into dentinal tubules decreases the interface between the core material and dentin, and retention of the core material might be improved by mechanical interlocking.[72]

In this study, maximum depth of penetration **(1080.94)** was observed in Isopropyl alcohol with BioRoot Sealer group (IIb) as compared to other groups and the mean depth of penetration was lowest for Roeko Surgi Tip Endo with resin-based sealer (Group IIIa) **(206.90).**

Considering the hydrophilic propensity of the calcium silicate-based sealer as compared to hydrophobic epoxy resin-based sealers, it may be speculated that isopropyl alcohol ($C_3H_7OH$), which has lower polarity than ethanol ($C_2H_5OH$), promoted less removal of the water from dentinal tubules, enhancing the dentin wettability, increasing the degree of conversion of the sealers and consequently improving their adhesion.[22,23]

In present study amongst all drying protocol BioRoot Sealer showed highest dentinal tubule penetration as compared to AH Plus sealer. Better results for BioRoot could be justified as BioRoot RCS showed higher calcium ion release than other sealers over a prolonged duration. The prolonged mineralizing ion release triggers the nucleation of calcium phosphate, which may improve the sealing ability of obturation materials.[62,73]

The results are in accordance to study of **Uzunoglu-Özyürek et al. (2018)[74]** where they stated that BioRoot RCS provided higher dentinal tubule penetration than AH 26, even in the presence of calcium hydroxide. Epoxy resin–based sealers have a neutral or acidic pH, whereas calcium silicate– based sealers have a basic pH. This parameter affects the penetration characteristics of the sealers, leading BioRoot RCS to have a higher penetration depth and percentage values.

Highly significant difference for depth of penetration in all groups was observed at coronal level **(p = < 0.0001,0.002)** for resin-based sealers and calcium silicate-based sealers respectively. Whereas, a non-significant difference in depth of penetration was observed in apical sections in all groups. **(p value = 0.106 ,0.138 )** for resin based sealers and calcium silicate based sealers respectively.

**Mjör A et al. (2000)**[75] reported the number of dentinal tubules to be about 40,000 per $mm^2$ in the coronal region of the root canal where as 14,400 per $mm^2$ in the apical region. Thus, a higher number and larger diameters of the tubules in the cervical and middle thirds could cause more sealer penetration in these areas as compared to apical third.

Another explanation for the decrease dentinal tubule penetration in apical third could be caused by different parameters such as tubular obliteration in the apical third, tapered root canal anatomy, limited accessibility of solutions to the most apical portions of the canal and difficulty to complete the removal of debris and the smear layer especially in the apical third.[76]

## II) Effect of drying protocol on push-out bond strength:

The presence or absence of humidity in the radicular dentin during the obturation process is a decisive factor for achieving an adequate filling. The bond strength between the sealer and the dentinal walls could be affected by the presence or absence of moisture, possibly leading to bond failures and leakage.

Higher values for bond strength were obtained for groups where Isopropyl Alcohol was used as a drying protocol as compared to those with paper point and Roeko surgitip -Endo, irrespective of the sections and sealers used.

These results are in accordance with the study done by **Dias KC et al. (2014)**[20] where they concluded that the bond strength of sealer to root dentin varies according to the drying protocol and that the drying with Isopropyl Alcohol significantly improves the bond strength of sealer to root dentin.

The results are also in accordance with what is shown by **Nagas et al. (2009)**[19] according to which all the sealer showed higher bond strengths under moisture conditions and lowest bond strength values under the ‘‘wet’’ condition as the hydrophilicity of sealers is never sufficient to displace water in a totally wet root canal, and the resultant entrapment of water droplets between the sealer dentin interface would lead to disruption of the bond. So, they suggested that it may be advantageous to leave the canals slightly moist before filling procedures.

Bio Root RCS showed the highest bond strength to root dentin in all moisture conditions when compared to AH Plus. **Uzunoglu et al. (2016)**[74] reported that there is a chemical bond with a micromechanical locking via cement tags in the dentinal tubule, which is referred to as the mineral infiltration zone, between calcium silicate cements and the dentin surface. From this it can be speculated that because of this interaction of calcium silicate-based sealer with the dentin surface, their adhesion to the root canal wall is better than that of epoxy resin-based sealer, which bonds to root canal wall via covalent bonds.

A highly significant difference in mean push-out bond strength were observed across groups as indicated by **p-value < 0.0001** at coronal, middle and apical levels. The highest mean push-out bond strength was seen in Group IIb **(4.53, 3.40, 2.95)** in coronal, middle and apical section respectively and lowest mean push-out bond strength value was seen in Group IIIa **(1.85,1.71,0.60)** in coronal, middle and apical section respectively.

Also, better results were obtained at coronal and middle thirds as compared to apical thirds as standardized degree of residual moisture may be difficult to achieve in all regions of the root. It happens because of differences in the dentinal tubule density and the limited accessibility of solutions to the most apical portions of the canal which may explain significant differences in the results observed among the canal thirds in some groups.[74,75]

## III) Correlation between intratubular penetration and push-out bond strength:

The depth of penetration and push out bond strengths for the two sealers used in this study differed after use of various drying protocols.

In the present study, statistically significant differences were observed between correlation of depth of sealer penetration and push out bond strength when three different drying protocols were used in both the sealers.

A higher value for intratubular penetration and push out bond strength was observed in the group where Isopropyl Alcohol was used as a drying protocol. Isopropyl alcohol ($C_3H_7OH$) has lower polarity, which promoted less removal of the water from dentinal tubules, enhancing the dentin wettability, increasing the depth of penetration of sealers and consequently improving their adhesion which ultimately also led to an increase in bond strength for both the sealers.[22,23]

Thus, showing a close relationship between intratubular penetration and push-out bond strength. There is limited research available on intratubular penetration and push-out bond strength of resin based and calcium silicate sealers after using various drying protocol and further more dedicated research is needed to be carried out in this area of correlation between intratubular penetration and Push out bond strength

Thus, the null hypothesis of this study that there would be no significant difference on Intratubular penetration and Push out bond strength of Resin-based sealer and calcium silicate-based after use of various drying protocols (Paper point, Isopropyl Alcohol and Roeko surgitip Endo) was rejected.

The intratubular penetration and push-out bond strength of the sealers differed significantly amongst the three groups.

Therefore, within the limitations of the study it can be concluded that Isopropyl alcohol has better drying and modifying root canal efficiency as compared to paper points and Roeko surgi-tip endo which are only physical methods of drying the canal. Isopropyl Alcohol showed significantly better intratubular penetration and greater push-out bond strength for Bio Root sealers.

# LIMITATIONS

Inspite of stringent care taken in every step of the root canal procedure certain limitations are present in the study

1. Even a slight variation in tooth dimensions can influence the results of the bond strength.

2. As this was an in vitro study, exact simulation of the oral conditions was not possible. Therefore, the results cannot be directly extrapolated to the clinical situation.

3. In this study a continually increasing static load was applied to the tooth which is not the type of load that occurs in natural oral environment.

# SUMMARY AND CONCLUSION

In the past decade, several experimental and clinical studies established that different levels of residual moisture in the root canal have shown to alter quality of adhesion between root canal dentin and sealers before filling procedures. However, several researchers have recommended various drying protocols to maintain root canals in a moist state and to improve the dentin hybridization of hydrophilic sealers.

The present in vitro study was carried out to evaluate the effect of various drying protocol (Paper point, Isopropyl Alcohol and Roeko surgitip-Endo) on Intratubular penetration and its relation to Push out bond strength of Resin based sealer and Calcium silicate-based sealer to root dentin.

In present study, 60 freshly extracted human mandibular first premolars, which fulfilled the inclusion criteria, were selected. The teeth were decoronated, prepared and irrigated using standard techniques.

After irrigation protocol, teeth were divided into three groups and three subgroups corresponding to treatment with different drying protocols and sealers used respectively.

## GROUP I:- PAPER POINT

Sub Group Ia:- Paper point with resin based sealer (n=10)

Sub Group Ib:- Paper point with calcium silicate based sealer(n= 10)

## GROUP II: - ISOPROPYL ALCOHOl

Sub Group IIa:- Isopropyl alcohol with resin based sealer( n= 10)

Sub Group IIb:- Isopropyl alcohol with calcium silicate based sealer(n=10)

## GROUP III:- ROEKO SURGITI- ENDO

Sub Group IIIa :- Roeko surgi tip – endo with resin based sealer(n=10)

Sub Group IIIb:- Roeko surgitip – endo with calcium silicate based sealer(n=10)

The samples were obturated with thermoplasticzed Obturation technique. After complete setting of both sealers, all samples were stored at 37 °C and 100% relative humidity for 1 week. All samples were then sectioned using a precision saw at 1,4 and 7 mm. These sections were evaluated for the depth of penetration under confocal laser scanning microscope and the same samples were evaluated for pushout bond strength under universal testing machine.

The results obtained indicated that there is a highly significant difference in depth of penetration and push-out bond strength when three different drying protocols were compared at coronal, middle and apical thirds. ($p$-value $<0.001$).

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

1. The depth of penetration and bond strength of resin based and calcium silicate-based sealers to root dentine varies according to the drying protocol.

2. Drying canals with Isopropyl Alcohol significantly improve the depth of penetration and bond strength of resin-based and calcium silicate-based sealers to root dentine.

3. The depth of penetration and push-out bond strength significantly decreased from cervical to apical level of root canal.

Taking into consideration the findings of the present study, it can be concluded that under experimental conditions, higher depth of penetration and bond strength of resin based and calcium silicate-based sealer to root dentine are present when the canals are dried with isopropyl alcohol. However, further investigations which could give a conclusive remark on the long-term effect of Isopropyl alcohol on the depth of penetration and bond strength of sealer to root dentin are needed.

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**Table 1:** Mean and standard deviation for *intratubular penetration* according to canal drying protocol and sealer at three tooth sections

| Tooth Section | Canal drying protocol | | | | | | | | | | | | P-value (Resin based sealer) | P-value (Calcium silicate) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Paper point | | | | Isopropyl alcohol | | | | Roeko surgitip-endo | | | | | |
| | Sealer | | | | Sealer | | | | Sealer | | | | | |
| | Resin based (n=10) | | Calcium silicate (n=10) | | Resin based (n=10) | | Calcium silicate (n=10) | | Resin based (n=10) | | Calcium silicate (n=10) | | | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | | |
| Coronal | 663.00 | 279.88 | 972.40 | 139.59 | 817.18 | 290.42 | 1080.94 | 403.68 | 206.90 | 56.93 | 570.37 | 290.30 | < 0.0001 (S) | 0.002 (S) |
| Middle | 329.30 | 171.28 | 441.60 | 153.31 | 402.30 | 160.39 | 674.81 | 374.71 | 184.00 | 33.85 | 263.80 | 94.10 | 0.005 (S) | 0.003 (S) |
| Apical | 141.50 | 54.85 | 253.00 | 105.29 | 193.60 | 150.93 | 319.12 | 217.65 | 100.70 | 29.77 | 175.40 | 119.87 | 0.106 (NS) | 0.138 (NS) |

SD: Standard deviation; P-value obtained using one-way analysis of variance; S: Significant; NS: Not significant

**Table 2a: Comparison of *intratubular penetration* between two sealer types for Paper point drying protocol at three sections**

| Tooth section | Paper point | | | | P-value* |
|---|---|---|---|---|---|
| | Sealer | | | | |
| | Resin based (n=10) | | Calcium silicate (n=10) | | |
| | Mean | SD | Mean | SD | |
| Coronal | 663.00 | 279.88 | 972.40 | 139.59 | 0.008 (S) |
| Middle | 329.30 | 171.28 | 441.60 | 153.31 | 0.140 (NS) |
| Apical | 141.50 | 54.85 | 253.00 | 105.29 | 0.011 (S) |

*Obtained using t-test for independent samples; S: Significant; NS: Not significant

**Table 2b: Comparison of *intratubular penetration* between two sealer types for Isopropyl alcohol drying protocol at three sections**

| Tooth section | Isopropyl alcohol | | | | P-value* |
|---|---|---|---|---|---|
| | Sealer | | | | |
| | Resin based (n=10) | | Calcium silicate (n=10) | | |
| | Mean | SD | Mean | SD | |
| Coronal | 817.18 | 290.42 | 1080.94 | 403.68 | 0.111 (NS) |
| Middle | 402.30 | 160.39 | 674.81 | 374.71 | 0.049 (S) |
| Apical | 193.60 | 150.93 | 319.12 | 217.65 | 0.153 (NS) |

*Obtained using t-test for independent samples; S: Significant; NS: Not significant

**Table 2c: Comparison of *intratubular penetration* between two sealer types for Roeko surgitip-endo drying protocol at three sections**

| Tooth section | Roeko surgitip-endo | | | | P-value* |
|---|---|---|---|---|---|
| | Sealer | | | | |
| | Resin based (n=10) | | Calcium silicate (n=10) | | |
| | Mean | SD | Mean | SD | |
| Coronal | 206.90 | 56.93 | 570.37 | 290.30 | 0.003 (S) |
| Middle | 184.00 | 33.85 | 263.80 | 94.10 | 0.028 (S) |
| Apical | 100.70 | 29.77 | 175.40 | 119.87 | 0.085 (NS) |

*Obtained using t-test for independent samples; S: Significant; NS: Not significant

**Table 3: Mean and standard deviation for *pushout bond strength* (MPa) according to canal drying protocol and sealer at three tooth sections**

| | Canal drying protocol | | | | | | | | | | | | P-value (Resin based sealer) | P-value (Calcium silicate sealer) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Paper point | | | | Isopropyl alcohol | | | | Roeko surgitip-endo | | | | | |
| | Sealer | | | | Sealer | | | | Sealer | | | | | |
| | Resin based (n=10) | | Calcium silicate (n=10) | | Resin based (n=10) | | Calcium silicate (n=10) | | Resin based (n=10) | | Calcium silicate (n=10) | | | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | | |
| Coronal | 2.67 | 0.48 | 3.74 | 0.78 | 3.18 | 0.95 | 4.53 | 1.39 | 1.59 | 0.64 | 2.12 | 0.77 | 0.001 (S) | < 0.0001 (S) |
| Middle | 2.25 | 0.36 | 2.75 | 0.67 | 2.37 | 0.76 | 3.40 | 0.66 | 1.50 | 0.60 | 2.04 | 0.60 | 0.045 (S) | < 0.0001 (S) |
| Apical | 1.73 | 0.43 | 1.84 | 0.47 | 1.97 | 0.66 | 2.95 | 0.61 | 0.60 | 0.36 | 0.84 | 0.49 | < 0.0001 (S) | < 0.0001 (S) |

SD: Standard deviation; P-value obtained using one-way analysis of variance; S: Significant; NS: Not significant

**Table 4a: Comparison of *pushout bond strength* between two sealer types for Paper point drying protocol at three sections**

| Tooth section | Paper point | | | | P-value* |
|---|---|---|---|---|---|
| | Sealer | | | | |
| | Resin based (n=10) | | Calcium silicate (n=10) | | |
| | Mean | SD | Mean | SD | |
| Coronal | 2.67 | 0.48 | 2.74 | 0.78 | 0.808 (NS) |
| Middle | 2.25 | 0.36 | 2.75 | 0.67 | 0.045 (S) |
| Apical | 1.73 | 0.43 | 1.84 | 0.47 | 0.586 (NS) |

*Obtained using t-test for independent samples; S: Significant; NS: Not significant

**Table 4b : Comparison of *pushout bond strength* between two sealer types for Isopropyl alcohol drying protocol at three sections**

| Tooth section | Isopropyl alcohol | | | | P-value* |
|---|---|---|---|---|---|
| | Sealer | | | | |
| | Resin based (n=10) | | Calcium silicate (n=10) | | |
| | Mean | SD | Mean | SD | |
| Coronal | 3.18 | 0.95 | 4.53 | 1.39 | 0.022 (S) |
| Middle | 2.37 | 0.76 | 3.40 | 0.66 | 0.040 (S) |
| Apical | 1.97 | 0.66 | 2.95 | 0.61 | 0.003 (S) |

*Obtained using t-test for independent samples; S: Significant

**Table 4c : Comparison of *pushout bond strength* between two sealer types for Roeko surgitip-endo drying protocol at three sections**

| Tooth section | Roeko surgitip-endo | | | | P-value* |
|---|---|---|---|---|---|
| | Sealer | | | | |
| | Resin based (n=10) | | Calcium silicate (n=10) | | |
| | Mean | SD | Mean | SD | |
| Coronal | 1.59 | 0.64 | 2.17 | 0.84 | 0.102 (NS) |
| Middle | 1.50 | 0.60 | 2.04 | 0.60 | 0.061 (NS) |
| Apical | 0.60 | 0.36 | 0.84 | 0.49 | 0.236 (NS) |

*Obtained using t-test for independent samples; NS: Not Significant

**Table 5: Correlation between intratubular penetration and pushout bond strength for each combination of canal drying protocol and sealer type**

| Pearson's correlation coefficient [P-value] | Canal drying protocol | | | | | |
|---|---|---|---|---|---|---|
| | Paper point | | Isopropyl alcohol | | Roeko surgitip-endo | |
| | Sealer | | Sealer | | Sealer | |
| | Resin based | Calcium silicate based | Resin based | Calcium silicate based | Resin based | Calcium silicate based |
| Intratubular penetration vs. Push out bond strength | 0.363 [0.047 (S)] | 0.380 [0.037 (S)] | 0.365 [0.047 (S)] | 0.403 [0.027 (S)] | 0.348 [0.059 (NS)] | 0.360 [0.048 (S)] |

S: Significant; NS: Not significant

.

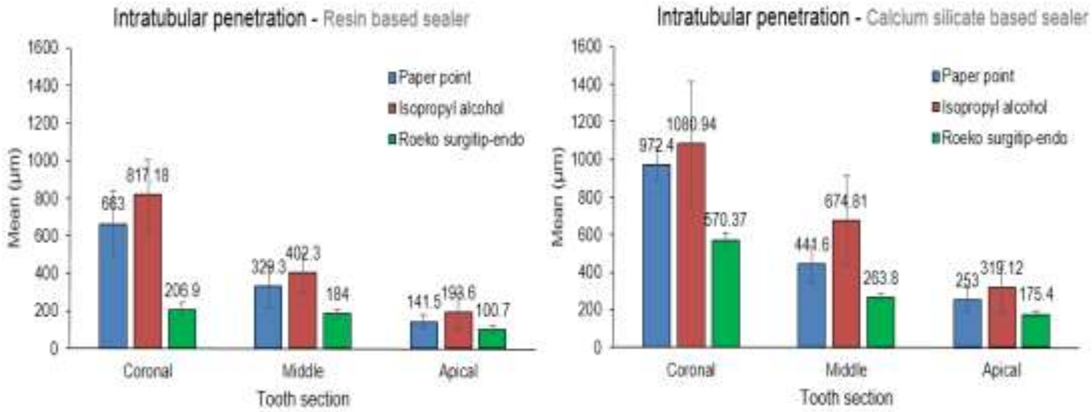


**Figure 1: Column chart showing mean intratubular penetration according sealer types for three canal drying protocols at each tooth section.**

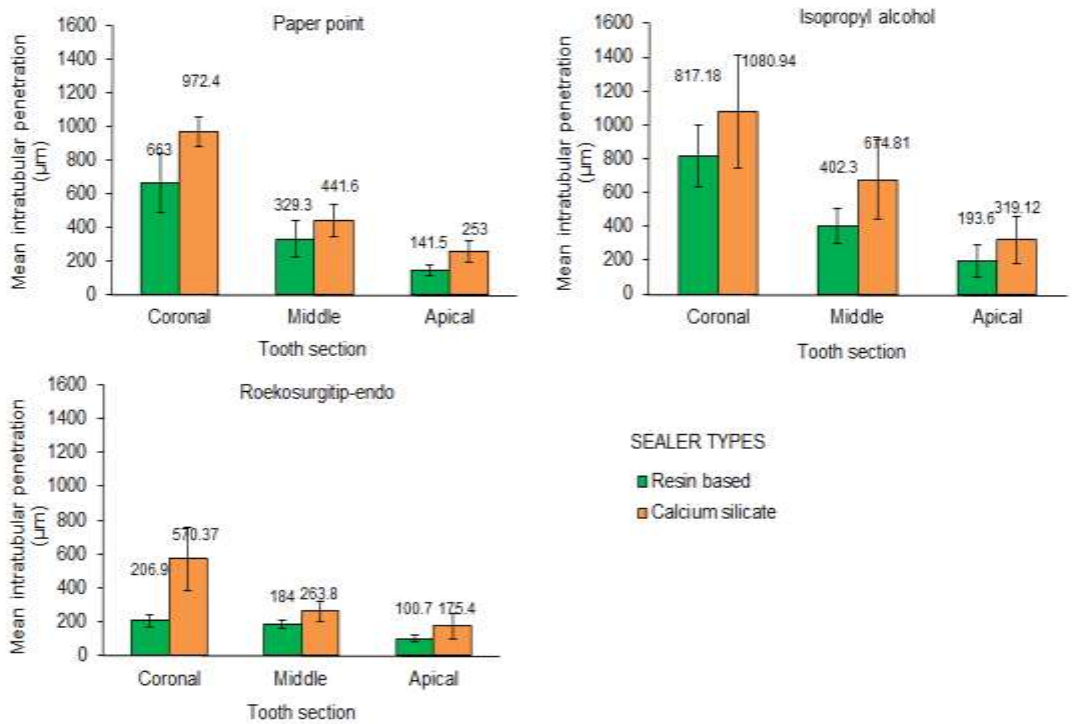


**Figure 2: Column chart showing mean intratubular penetration according to canal drying protocols and two sealer types at each section.**

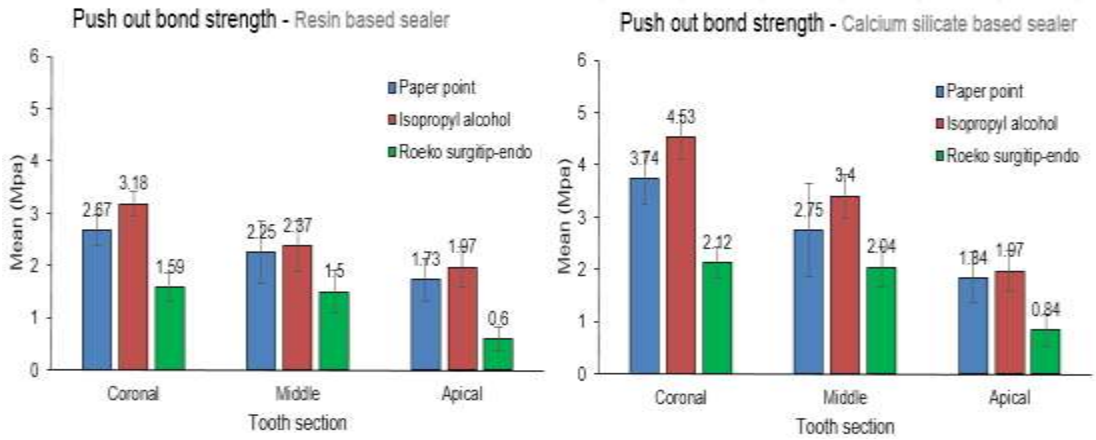


**Figure 3: Column chart showing mean push out bond strength according sealer types for three canal drying protocols at each tooth section.**

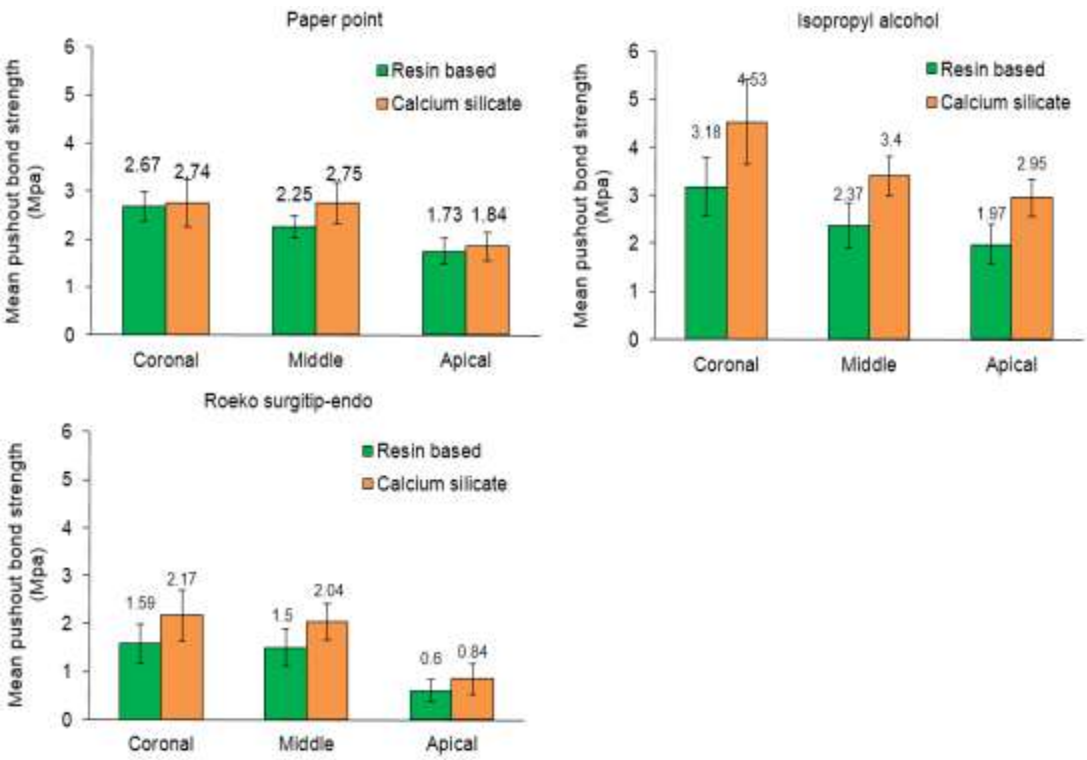


**Figure 4: Column chart showing mean pushout bond strength according to canal drying protocols and two sealer types at three tooth sections.**

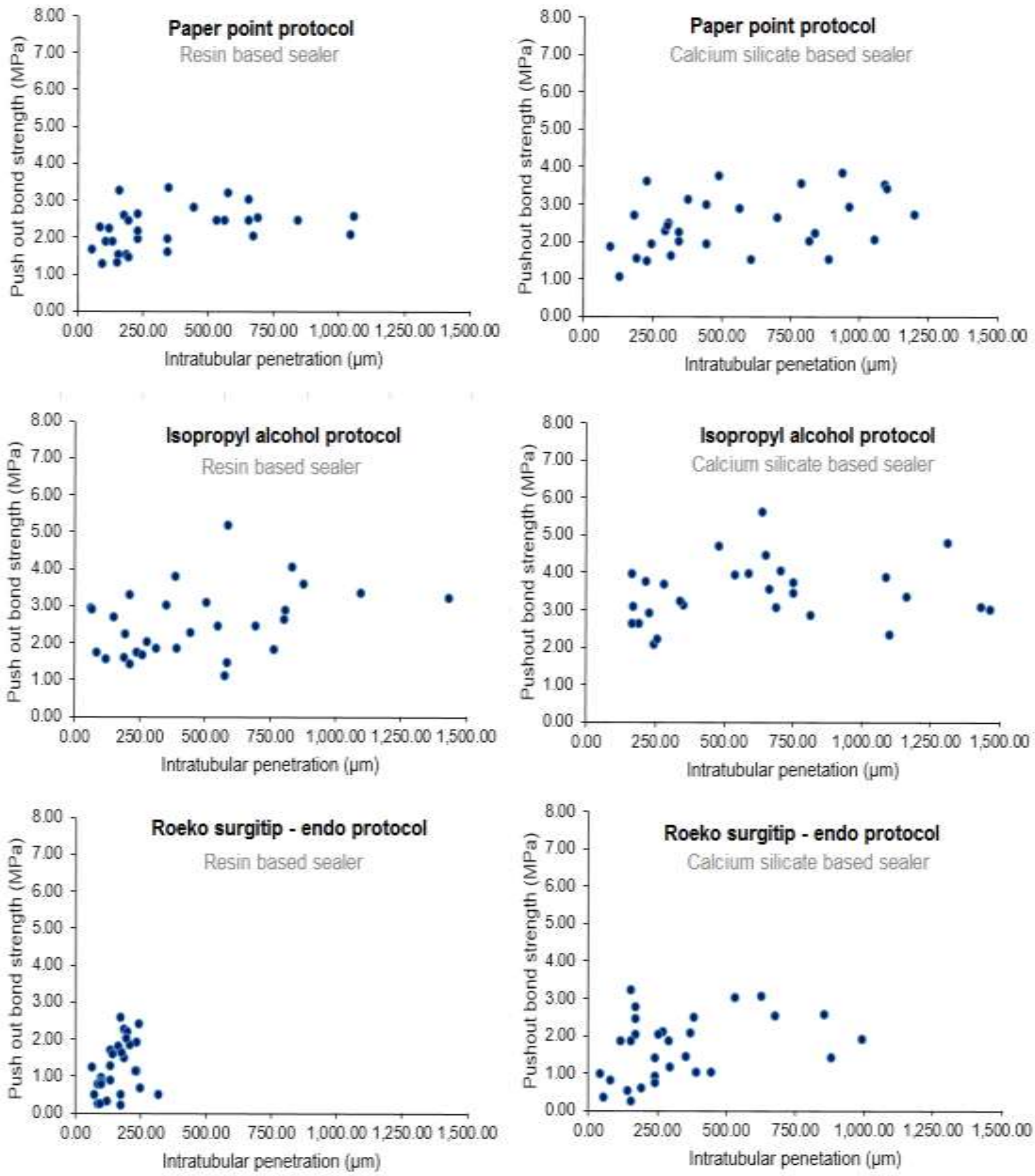


**Figure 5: Scatter plots showing relationship of intratubular penetration and pushout bond strength for various combinations of canal drying protocols and sealers.**

# ANNEXURE I

## Intratubular sealer penetration in Group Ia for each section.

| **Group Ia : Paper point with resin based sealer** | | | |
|---|---|---|---|
| **Sample no.** | **Coronal** | **Middle** | **Apical** |
| | **Depth of penetration** | **Depth of penetration** | **Depth of penetration** |
| 1 | 845Կm | 445Կm | 109Կm |
| 2 | 161Կm | 234Կm | 98Կm |
| 3 | 567Կm | 123Կm | 56Կm |
| 4 | 349Կm | 657Կm | 189Կm |
| 5 | 675Կm | 345Կm | 134Կm |
| 6 | 1047Կm | 345Կm | 89Կm |
| 7 | 1059Կm | 534Կm | 232Կm |
| 8 | 657Կm | 234Կm | 198Կm |
| 9 | 580Կm | 178Կm | 156Կm |
| 10 | 690Կm | 198Կm | 154Կm |

# ANNEXURE II

## Intratubular sealer penetration in Group I b for each section.

| Group I b : Paper point with calcium silicate based sealer | | | |
|---|---|---|---|
| **Sample no** | **Coronal** | **Middle** | **Apical** |
| | **Depth of penetration** | **Depth of penetration** | **Depth of penetration** |
| 1. | 840µm | 708µm | 135µm |
| 2. | 1100µm | 567µm | 198µm |
| 3. | 966µm | 303µm | 250µm |
| 4. | 946µm | 229µm | 304µm |
| 5. | 1205µm | 382µm | 450µm |
| 6. | 1061µm | 313µm | 189µm |
| 7. | 1102µm | 452µm | 102µm |
| 8. | 790µm | 612µm | 231µm |
| 9. | 824µm | 352µm | 350µm |
| 10. | 890µm | 498µm | 321µm |

# ANNEXURE III

## Intratubular sealer penetration in Group IIa for each section

| **Group IIa : Isopropyl alcohol with Resin based sealer** | | | |
|---|---|---|---|
| **Sample no.** | **Coronal** | **Middle** | **Apical** |
| | **Depth of penetration** | **Depth of penetration** | **Depth of penetration** |
| 1. | 811.9ɥm | 588ɥm | 85ɥm |
| 2. | 878.9ɥm | 315ɥm | 63ɥm |
| 3. | 804ɥm | 697ɥm | 579ɥm |
| 4. | 765ɥm | 390ɥm | 215ɥm |
| 5. | 590ɥm | 356ɥm | 262ɥm |
| 6. | 836ɥm | 213ɥm | 192ɥm |
| 7. | 444ɥm | 280ɥm | 197ɥm |
| 8. | 1098ɥm | 392ɥm | 68ɥm |
| 9. | 1438ɥm | 553ɥm | 152ɥm |
| 10. | 506ɥm | 239ɥm | 123ɥm |

**ANNEXURE IV**

## Intratubular sealer penetration in Group IIB for each section.

| Group IIb : Isopropyl alcohol with calcium silicate based sealer | | | |
|---|---|---|---|
| Sample no. | Coronal | Middle | Apical |
| | Depth of penetration | Depth of penetration | Depth of penetration |
| 1. | 1504.98Կm | 1437.98Կm | 252.3Կm |
| 2. | 1090. 59Կm | 354.68Կm | 263.24Կm |
| 3. | 646.39Կm | 1106.9Կm | 287.02Կm |
| 4. | 594.9Կm | 669.48Կm | 231.34Կm |
| 5. | 1317.98Կm | 819.2Կm | 192.14Կm |
| 6. | 653.27Կm | 220.38Կm | 167.25Կm |
| 7. | 1165.9Կm | 547.21Կm | 178.3Կm |
| 8. | 711.28Կm | 489.86Կm | 172.11Կm |
| 9. | 1473.2Կm | 346.76Կm | 692.84Կm |
| 10. | 1650.87Կm | 755.65Կm | 754.67Կm |

# ANNEXURE V

## Intratubular sealer penetration of Group IIIa for each section.

| Group III a: Roeko surgi tip- endo with resin based sealer | | | |
|---|---|---|---|
| **Sample no.** | **Coronal** | **Middle** | **Apical** |
| | **Depth of penetration** | **Depth of penetration** | **Depth of penetration** |
| 1. | 250μm | 173μm | 101μm |
| 2. | 189μm | 143μm | 174μm |
| 3. | 321μm | 165μm | 121μm |
| 4. | 135μm | 234μm | 86μm |
| 5. | 243μm | 134μm | 67μm |
| 6. | 189μm | 209μm | 75μm |
| 7. | 234μm | 198μm | 89μm |
| 8. | 200μm | 175μm | 95μm |
| 9. | 174μm | 231μm | 101μm |
| 10. | 134μm | 178μm | 98μm |

# ANNEXURE VI

## Intratubular sealer penetration of Group IIIb for each section.

| Group IIIb : Roeko surgi tip -endo with calcium silicate based sealer | | | |
|---|---|---|---|
| **Sample No.** | **Coronal** | **Middle** | **Apical** |
| | **Depth of penetration** | **Depth of penetration** | **Depth of penetration** |
| 1. | 301.21Կm | 388Կm | 56Կm |
| 2. | 246.5Կm | 356Կm | 452Կm |
| 3. | 178.03Կm | 393Կm | 245Կm |
| 4. | 376.09Կm | 175Կm | 123Կm |
| 5. | 888.32Կm | 156Կm | 45Կm |
| 6. | 1001Կm | 160Կm | 87Կm |
| 7. | 864Կm | 278Կm | 198Կm |
| 8. | 537.8Կm | 178Կm | 156Կm |
| 9. | 679.9Կm | 298Կm | 243Կm |
| 10. | 630.89Կm | 256Կm | 148Կm |

# ANNEXURE VII

## Push out bond strength of sealer to root dentin in group Ia for each section

| Group Ia: Paper Point AH Plus | | | | | | |
|---|---|---|---|---|---|---|
| Sr. No. | Coronal | | Middle | | Apical | |
| | Push out Force (N) | Push out Bond Strength (MPa) | Push out Force (N) | Push out Bond Strength (MPa) | Push out Force (N) | Push out Bond Strength (MPa) |
| 1 | 14.95 | 2.43 | 14.65 | 2.78 | 8.95 | 1.88 |
| 2 | 20.00 | 3.25 | 10.15 | 1.93 | 6.00 | 1.26 |
| 3 | 14.90 | 2.42 | 11.17 | 2.22 | 7.80 | 1.64 |
| 4 | 20.56 | 3.34 | 12.87 | 2.44 | 4.67 | 0.97 |
| 5 | 12.43 | 2.02 | 4.98 | 0.94 | 8.96 | 1.87 |
| 6 | 11.34 | 1.84 | 8.90 | 1.6 | 10.78 | 2.25 |
| 7 | 15.65 | 2.54 | 12.87 | 2.44 | 12.43 | 2.60 |
| 8 | 18.43 | 2.99 | 11.34 | 2.15 | 4.67 | 0.97 |
| 9 | 19.54 | 3.17 | 13.67 | 2.59 | 7.32 | 1.53 |
| 10 | 15.45 | 2.51 | 12.87 | 2.44 | 6.24 | 1.30 |

# ANNEXURE VIII

## Push out bond strength of Sealer to Root dentin in Group Ib for each section.

| Group Ib: Paper Point Bio Root | | | | | | |
|---|---|---|---|---|---|---|
| Sr. No. | Coronal | | Middle | | Apical | |
| | Push out Force (N) | Push out Bond Strength (MPa) | Push out Force (N) | Push out Bond Strength (MPa) | Push out Force (N) | Push out Bond Strength (MPa) |
| 1 | 30.80 | 5.00 | 13.80 | 2.62 | 4.95 | 1.04 |
| 2 | 21.40 | 3.48 | 15.15 | 2.87 | 7.30 | 1.53 |
| 3 | 17.80 | 2.89 | 11.90 | 2.26 | 9.10 | 1.91 |
| 4 | 23.43 | 3.80 | 18.90 | 3.58 | 11.54 | 2.41 |
| 5 | 16.45 | 2.67 | 16.45 | 3.12 | 11.76 | 2.46 |
| 6 | 12.43 | 2.02 | 12.98 | 2.46 | 12.87 | 2.69 |
| 7 | 20.76 | 3.37 | 15.67 | 2.97 | 8.90 | 1.86 |
| 8 | 21.63 | 3.51 | 14.98 | 2.84 | 6.98 | 1.46 |
| 9 | 20.78 | 3.37 | 11.77 | 2.23 | 9.54 | 2 |
| 10 | 26.87 | 4.36 | 19.76 | 3.74 | 7.67 | 1.60 |

# ANNEXURE IX

## Push out bond strength of Sealer to root dentin in Group IIa for each section

| Group IIa: Isopropyl Alcohol AH Plus | | | | | | |
|---|---|---|---|---|---|---|
| Sr. No. | Coronal | | Middle | | Apical | |
| | Push out Force (N) | Push out Bond Strength (MPa) | Push out Force (N) | Push out Bond Strength (MPa) | Push out Force (N) | Push out Bond Strength (MPa) |
| 1 | 5.15 | 0.84 | 7.65 | 1.45 | 8.20 | 1.72 |
| 2 | 21.90 | 3.56 | 9.64 | 1.82 | 13.95 | 2.93 |
| 3 | 16.10 | 2.62 | 12.76 | 2.42 | 5.10 | 1.07 |
| 4 | 4.90 | 0.79 | 19.86 | 3.76 | 6.78 | 1.42 |
| 5 | 31.75 | 5.16 | 15.72 | 2.98 | 7.93 | 1.66 |
| 6 | 24.80 | 4.03 | 17.32 | 3.28 | 7.54 | 1.58 |
| 7 | 13.89 | 2.25 | 10.67 | 2.02 | 10.65 | 2.23 |
| 8 | 20.32 | 3.30 | 9.62 | 1.82 | 13.76 | 2.88 |
| 9 | 19.65 | 3.19 | 12.76 | 2.42 | 12.87 | 2.69 |
| 10 | 18.98 | 3.08 | 8.98 | 1.71 | 7.34 | 1.53 |

# ANNEXURE X

## Push Out bond strength of sealer to Root dentin in group IIb for each section.

| Group IIb: Isopropyl Alcohol BioRoot | | | | | | |
|---|---|---|---|---|---|---|
| Sr. No. | Coronal | | Middle | | Apical | |
| | Push out Force (N) | Push out Bond Strength (MPa) | Push out Force (N) | Push out Bond Strength (MPa) | Push out Force (N) | Push out Bond Strength (MPa) |
| 1 | 37.65 | 7.89 | 6.05 | 1.14 | 5.04 | 1.05 |
| 2 | 18.30 | 3.84 | 16.45 | 3.12 | 10.56 | 2.21 |
| 3 | 26.60 | 5.58 | 12.10 | 2.29 | 17.54 | 3.67 |
| 4 | 24.25 | 3.94 | 18.67 | 3.54 | 13.76 | 2.88 |
| 5 | 29.15 | 4.74 | 14.87 | 2.82 | 12.45 | 2.61 |
| 6 | 27.20 | 4.42 | 19.64 | 3.72 | 18.93 | 3.96 |
| 7 | 20.38 | 3.31 | 20.56 | 3.90 | 14.67 | 3.07 |
| 8 | 24.65 | 4.00 | 24.76 | 4.69 | 12.46 | 2.61 |
| 9 | 18.34 | 2.98 | 16.89 | 3.20 | 14.56 | 3.05 |
| 10 | 28.57 | 4.64 | 19.56 | 3.71 | 16.32 | 3.42 |

# ANNEXURE XI

## Push out bond strength of sealer to root dentin in Group IIIa for each section.

| Group IIIa: Roeko Endo AH Plus | | | | | | |
|---|---|---|---|---|---|---|
| Sr. No. | Coronal | | Middle | | Apical | |
| | Push out Force (N) | Push out Bond Strength (MPa) | Push out Force (N) | Push out Bond Strength (MPa) | Push out Force (N) | Push out Bond Strength (MPa) |
| 1 | 4.10 | 0.67 | 13.50 | 2.56 | 4.60 | 0.96 |
| 2 | 13.80 | 2.24 | 8.35 | 1.58 | 10.55 | 0.21 |
| 3 | 16.10 | 3.05 | 9.45 | 1.79 | 1.40 | 0.29 |
| 4 | 6.67 | 1.26 | 5.89 | 1.11 | 3.76 | 0.78 |
| 5 | 14.87 | 2.41 | 4.67 | 0.88 | 5.78 | 1.21 |
| 6 | 7.76 | 1.47 | 9.67 | 1.83 | 2.34 | 0.49 |
| 7 | 11.75 | 1.91 | 10.56 | 2.00 | 1.08 | 0.22 |
| 8 | 13.54 | 2.17 | 13.98 | 2.65 | 5.89 | 0.23 |
| 9 | 8.65 | 1.64 | 5.98 | 1.13 | 8.76 | 0.83 |
| 10 | 10.32 | 1.67 | 8.56 | 1.62 | 3.76 | 0.78 |

# ANEXURE XII

## Push out bond strength of sealer to root dentin in group IIIb for each section.

| Group IIIb: Roeko Endo Bio Root | | | | | | |
|---|---|---|---|---|---|---|
| Sr. No. | Coronal | | Middle | | Apical | |
| | Push out Force (N) | Push out Bond Strength (MPa) | Push out Force (N) | Push out Bond Strength (MPa) | Push out Force (N) | Push out Bond Strength (MPa) |
| 1 | 7.00 | 1.14 | 13.10 | 2.49 | 1.60 | 0.34 |
| 2 | 5.45 | 0.89 | 7.60 | 1.44 | 4.70 | 0.99 |
| 3 | 16.85 | 2.74 | 5.40 | 1.02 | 6.60 | 1.38 |
| 4 | 12.56 | 2.04 | 10.67 | 2.02 | 8.90 | 1.86 |
| 5 | 8.65 | 1.40 | 9.65 | 1.83 | 4.65 | 0.97 |
| 6 | 10.86 | 1.76 | 16.87 | 3.20 | 3.87 | 0.81 |
| 7 | 15.76 | 2.56 | 10.98 | 2.08 | 2.76 | 0.57 |
| 8 | 18.54 | 3.01 | 12.89 | 2.44 | 1.09 | 0.22 |
| 9 | 19.34 | 3.14 | 9.67 | 1.83 | 3.54 | 0.74 |
| 10 | 18.65 | 3.03 | 10.67 | 2.02 | 2.43 | 0.50 |