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"Influence of Different Irrigation Sequence on Penetration of Root Canal Sealers to Dentin- A Confocal Laser Scanning Microscopic Study"

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ABSTRACT
Aim:*To evaluate the influence of different irrigation sequence on penetration of root canal sealers to dentin.*
Materials and methods*: A total of one hundred and twenty human single rooted mandibular premolars were selected anddecoronated at the cemento-enamel junction. Instrumentation done in all the canals using Protaper Universal Rotary files upto F3. The specimens were randomly divided into four experimental groups based on irrigation sequence followed. In Group I 3% NaOCl followed by 17% EDTA, Group-II17% EDTA followed by 3% NaOCl, Group III 17% EDTA followed by 2% chlorhexidine and In Group IV 3% NaOCl followed by Normal saline. Then again based on sealer used each group is sub divided into 3 subgroups. Sub group A- AH Plus, Sub group B- Apexit, Sub group C- MTA Fillapex, All the canal were obturated with cold lateral compaction. Teeth were sectioned horizontally perpendicular to the long axis of teeth at 4, 8 and 12mm from apex. These sections were examined under CLSM at X10 magnification to determine depth of sealer penetration.*
Results*:Sealer penetration is significantly higher for when EDTA followed by Sodium hypochlorite (group- II) is used as final irrigation protocol.(mean penetration = 559.44µm).*
Conclusions:*Final rinse with 3% NaOCl, after a rinse with 17% EDTA influenced the maximum depth of the sealer penetration.*
Key words: *CHX- Chlorhexidine, CLSM- Confocal Laser Scanning Microscopy, EDTA-Ethylenediaminetetraacetic acid,.*

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I. Introduction

The endodontic triad for successful root canal therapy comprises shaping and cleaning, disinfection followed by three-dimensional (3D) obturation of the root canal system. (1) The purposes of root canal treatments are to eliminate microorganisms from the root canal system and to prevent re-contamination. Because of the complex anatomy of the root canal system, which includes lateral canals, ramifications, and deltas, it is impossible to complete disinfection of the root canal using instrumentation alone (2). Irrigation is a critical compliment to instrumentation because it removes bacteria, debris, and necrotic tissue present in the smear layer. The smear layer hinders the penetration of intracanal medicaments and sealers into the dentinal tubules, thus protecting bacteria within the dentinal tubules. Chelating agents, such as EDTA and combinations of EDTA and NaOCl have been used to remove the smear layer. (2) Final irrigation protocol followed is of paramount importance in achieving complete disinfection within the root canal, and also the penetration of sealer serves as an indicator of the extent to which the smear layer was removed. (1)

Many studies found that a poorly filled canal as the most common cause of failure of root canal treatment. The success of the root canal treatment and periapical health and healing depend on the quality of root canal filling.(3) The standard root filling is a combination of sealer cement with a central core material, which until now has been almost exclusively gutta-percha. The core acts as a piston on which the flowable sealer, causing it to spread, fill voids and to wet and attach to the instrumented dentin wall. The sealers are responsible for the sealing off of the root canal system, entombment of remaining bacteria and the filling of irregularities in the prepared canal which cannot be filled by gutta-percha.(4)

There are various kinds of sealers used depending upon the combination, constituents in clinical practice. However, in this study three types of sealers are used namely Resin based sealers, Calcium hydroxide based sealers, MTA based sealers.

By far the most successful of the resin-based sealers has been the AH series. AH Plus consists of a paste–paste system, delivered in two tubes in a new double barrel syringe. The epoxide paste contains radiopaque fillers and aerosil. The amine paste consists of three different types of amines, radiopaque fillers, and aerosil. AH Plus has better penetration into the microirregularities because of its creep capacity and long setting time, which increases the mechanical interlocking between sealer and root dentin and the cohesion of sealer causes Resin to be more resistant to fracture (5).

The success of calcium hydroxide as a pulp protecting and capping agent and as an interappointment dressing prompted its use also in sealer cement formulations. Apexit is well known brand name of this type of material (4). The two most important reasons for using calcium hydroxide as a root-filling material are stimulation of the periapical tissues in order to maintain health or promote healing and secondly for its antimicrobial effects(5).The strong interest in developing mineral trioxide aggregate (MTA)-based endodontic materials is because of the excellent biocompatibility, bioactivity, and osteoconductivity of MTA. MTA Fillapex is a sealer that is composed of MTA, salicylate resin, natural resin, bismuth oxide, and silica. A recent study showed that this sealer has suitable physicochemical properties, such as good radiopacity, flow, and alkaline pH. The manufacturer states that it has a great working time, low solubility, and easy handling. (6)

Several microscopy techniques are currently used to evaluate the sealer/dentin interface, including stereomicroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and confocal laser scanning microscopy (CLSM) .In comparison to conventional SEM, confocal microscopy has the advantage of providing detailed information about the presence and distribution of sealers or dental adhesives inside dentinal tubules in the total circumference of the root canal walls at relative low magnification as 10X through the use of fluorescent Rhodamine marked sealers.(7)

Various methods like shear bond strength, microtensile bond strength and push-out bond strength are used to examine the adhesive strength of the root canal sealers. The push-out bond test has been widely performed to measure the bond strength of numerous root canal sealers, but, the results have been inconsistent (8). Moreover, there are no reports of studies on that have evaluated the influence of different irrigation sequence on penetration of root canal sealers to dentin by using confocal laser scanning microscope.

Hence the present study aim is to evaluate influence of different irrigation sequence on penetration of root canal sealers to dentin by using confocal laser scanning microscope.

II. Materials And Methodology

MATERIALS

1. 120 Single Rooted Mandibular premolars
2. Micromotor (SaeyangMicrotech, South Korea)
3. #10 k, #15 k-file (Mani Inc, Japan)
4. 3% NaOCl (Vishal Dentocare PVT. LTD, Gujarat, India)
5. 2% chlorhexidine (Vishal Dentocare PVT. LTD, Gujarat, India)
6. 17% EDTA (Ramen Research Products ,Kolkata,India)
7. Distilled water (Sun Life Pharma Services,India)
8. Saline (Braun Group Company , India)
9. Paper points (Gapadent,IDS , Dentmep PVT LTD,India)
10. Xsmart (Dentsply Tulsa Dental Specialities, USA)
11. Protaper Universal Rotary files (DentsplyMaillefer, Ballaigues, Switzerland)
12. Root Cansal Sealers – (AH plus sealer (Dentsply India PVT.LTD, Delhi, India), Apexit (Ivoclar ,Vivadent), MTA Fillapex (Angelus, Londrina, Brazil)
13. Rhodamine B Dye (SDFCL,Mumbai,India)
14. Lentulo spirals (Mani Inc, Japan)
15. Confocal laser scanning microscope (ZEISS LSM,700, Germany)

METHODOLOGY

Sample preparation

A total of one hundred and twenty human extracted single rooted mandibular premolars without fracture lines or caries were selected. After extraction, the teeth were carefully cleaned to eliminate tartar and tissue remains, followed by storage in a dilute solution of NaOCl (Sodium hypochlorite) for one week so as to remove any remaining organic debris. The teeth were decoronated at the cemento-enamel junction, at low speed under water cooling to prevent overheating. A #10 K-file was used to negotiate the canal and working length was standardized up to 15mm using #15 K-file. Instrumentation is done in all the canals using Protaper Universal Rotary files upto F3 in crown down manner and were irrigated with 5 ml of NaOCl followed by followed by final flushing with 5 ml saline solution to remove any remnants. All the canals dried with paper points.(1)

The specimens were randomly divided into four experimental groups based on irrigation sequence followed: with each group containing 30 specimens(n=30).
In Group I the specimens were first irrigated with 5ml of 3% NaOCl followed by 17% EDTA.
In Group II the specimens were first irrigated with 5ml of 17% EDTA followed by 3% NaOCl.
In Group III the specimens were first irrigated with 5ml of 17% EDTA followed by 2% chlorhexidine.
In Group IV the specimens were first irrigated with 5ml of 3% NaOCl followed by Normal saline.
Distilled Water is used in between each irrigant . All irrigation solutions were introduced in to the canal using 5ml disposable plastic syringe. Final rinse of 5 ml of distilled water used for all groups. Then again based on sealer used each group is sub divided into 3 subgroups: with each sub group containing 10 specimens (n=10).
In sub group A all the specimens coated with AH Plus sealer
In sub group B all the specimens coated with Apexit sealer
In sub group A all the specimens coated with MTA Fillapex sealer

All Sealers were labelled with Rhodamine B dye and are mixed on glass slab. The sealer was introduced into all the canals by means of lentulo spirals 25 to 1 mm short of the WL in a pumping motion for 5 seconds. All the canal were obturated with cold lateral compaction and then teeth were stored in an incubator at 37˚C and 100% humidity for 7 days to allow the sealer to set. Teeth were sectioned horizontally perpendicular to the long axis of teeth at 4, 8 and 12mm from apex representing apical, middle, coronal thirds respectively using hard tissue microtome under continuous water supply. These sections were examined under CLSM (ZEISS LSM 700, Germany) at X10 magnification. The depth of sealer penetration into dentinal tubules was calculated as the average penetration measured, by using the Zeiss LSM Image Examiner software (Carl Zeiss).(1)

III. Results

According to results obatained table -1 shows the sealer penetration is significantly higher for when EDTA followed by Sodium hypochlorite (group- II) is used as final irrigation protocol.(mean penetration = 559.44μm). There is statistically significant difference in mean sealer penetration when Group- I (mean penetration = 556.89μm) compared with Group- III (mean penetration = 357.67μm) and Group- IV (mean penetration = 219.22μm) also there is statistically significant difference when Group- II is compared with Group- III and Group- IV. There is no significant difference between Group- I and Group- II.(Table -2, Graph-1).

When type of sealer is concerned AH plus sealer (mean penetration = 508.83μm) has significantly higher penetration efficacy followed by Apexit (mean penetration = 426.67μm) and least penetration observed with MTA Fillapex (mean penetration = 334.42μm) irrespective of the irrigation protocol used..(Table -1, Graph- 1).

While observing root third coronal third (mean penetration = 658.17μm) has significantly higher sealer penetration when compared to middle third (mean penetration = 428.25μm) and apical third (mean penetration = 183.50 μm) in all the experimental groups.(Table -3, Graph- 2).

STATISTICAL ANALYSIS:

Statistical analysis was done using three-way ANOVA and Tukey's multiple post hoc procedures with SPSS 20.0 software for comparison of root canal sealer penetration among various irrigation protocols.

Table – 2 shows Comparison of root canal sealer penetration among various irrigation protocols. There is no significant difference when Group- I compared with Group- II with a P value ($P > 0.05$). But there is significant difference when Group- I and Group- II are compared with Group- III and Group- IV with a P value ($P < 0.05$).

Table – 3 shows Comparison of sealer penetration among root thirds coronal third has statistically significant difference when compared to middle third and apical third with P value ($P < 0.05$).(Graph- 2)

Table – 4 shows Comparison of sealer penetration among 3 sealers.AH Plus has statistically significant difference when compared to Apexit and MTA Fillapex with P value ($P < 0.05$).(Graph- 1)

IV. Discussion

The success of endodontic treatment depends largely on shaping and cleaning, disinfection, and 3D obturation. An age-old adage is that: "Files shape while irrigants clean." The primary aim of endodontic treatment is to eliminate all debris and microorganisms from the root canal system to prevent recontamination. The complex anatomy of the root canal system makes it impossible to completely disinfect the root canal using instrumentation alone.(1)

Instrumentation during root canal therapy produces an amorphous, granular, and irregular layer covering dentin, known as smear layer. This consists of inorganic debris and organic components, such as pulp tissue remnants, odontoblastic processes, saliva, blood cells, and bacteria (1), (9). Smear layer prevents the penetration of intracanal medicaments into dentinal tubules and influences the adaptation of filling materials to canal wall. (10)

Irrigation is an essential part of root canal debridement. It allows for cleaning beyond what might be achieved by root canal instrumentation alone. It helps by killing microorganisms, flushing debris, and removing the smear layer from the root canal system (1),(10). Irrigation solutions, such as sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) and chlorhexidine should be used for superior disinfection during canal preparation (11).

Among the irrigants, NaOCl solution is considered the gold standard because of its exceptional qualities as an antiseptic, its tissue dissolving effects and its excellent antimicrobial effects. Although NaOCl appears to be the most desirable single endodontic irrigant, it cannot dissolve the inorganic components of the smear layer. Therefore, NaOCl has been used in combination with EDTA for effective removal of the smear layer(12).

Smear layer removal from root canal walls during instrumentation allows endodontic irrigants and filling materials to enter the dentinal tubules. For effective removal of smear layer, judicious use of chelating agents such as EDTA is of paramount importance .The alternate use of sodium hypochlorite (NaOCl), a deproteinizing agent, and ethylenediaminetetraaceticacid (EDTA), a calcium-chelating agent, has been recommended for its efficient removal .These irrigants must be brought into direct contact with the entire canal wall for effective action (1),(10)

The main objective of a root canal filling is to seal the root canal system to prevent reinfection. Normally, a root canal filling is associated with a hard core, like gutta-percha, and a sealer to better adapt the root canal filling material and complete the seal of the root canal filling in the most effectual manner .Therefore, the sealer root canal wall interface is crucial for the sealing of the root canal system . The sealer can fill the irregularities of the root canal wall and the dentinal tubules, which cannot be filled by gutta-percha. Sealer penetration into the tubules could affect the seal of the root filling because an increase of the contact surface between filling material and dentin is related to an improvement of the sealability. Also, sealer penetration can promote an antimicrobial effect in the tubules, which increases when in closer contact with the microbes.(1),(4)

So Sealer penetration might serve as an indicator of the extent to which the smear layer was removed (Oksanet al., 1993, Kouvaset al.,1998,Kokkaset al., 2004).(13),(14).Sealer penetration into dentinal tubules is considered a positive outcome to prevent bacterial repopulation or bacterial inactivation inside the tubules as a blocking agent, improve filling material retention within the root canal. Therefore, sealer penetration into dentinal tubules is considered clinically relevant. Penetration of the root canal sealer into the dentinal tubule can provide a mechanical interlocking between the sealer and root dentin. On the other hand, varied physical and chemical properties of the sealer and different irrigant solutions can influence the depth of penetration. (2),(15).

Many studies have reported the penetration of sealer into root canal dentin. To analyze the extent of sealer penetration, SEM, light microscopy, and CLSM have been used. In the present study, CLSM was used because this technique has several advantages over SEM. In comparison with SEM and histologic methods, confocal microscopy has the advantage of providing detailed information about the presence and distribution of sealers or dental adhesives inside dentinal tubules in the total circumference of the root canal walls at magnification as low as 50X to 100X through the use of fluorescent dye-marked sealers. The principal advantage of CLSM is that it allows the study of a volume of dentin in non dehydrated specimens at the subsurface level of specimens using histomographic images.(2),(16).

Root canal sealers have no fluorescent properties; hence, it was required to add a fluorescent rhodamine B dye to the sealers to allow visualization under the CLSM. The sealer labelled with 0.1% rhodamine did not show changes in flow according to American Dental Association specifications. Pilot studies were performed to determine whether labelled sealer and unlabelled sealer had different physicochemical properties. It was concluded that the sealer labelled with 0.1% rhodamine did not show changes in flow according to American Dental Association specifications.(2)

In the present study 5ml distilled water used as final flush because this can effectively remove innate calcium ion in the dentin which might influence the penetration depths of the sealer after obturation.(16)

In NaOCl/EDTA protocol the specimens were first irrigated with 5ml of 3% NaOCl followed by 17% EDTA and finally rinsed with 5ml of distilled water. According to the results of this study in this group AH Plus shows higher mean sealer penetration than Apexit and MTA Fillapex. The combination of NaOCl and EDTA completely removed the smear layer and left a smooth, planed surface with a patent tubular orifices. This results in more sealer penetration in NaOCl/ETDA irrigation protocol. So combination of NaOCl and EDTA is more preferred rather than using NaOCl or EDTA alone (18). According to the results of this study depth of sealer penetration was highest when final irrigation was ssfact that EDTA reacts with the calcium ions in dentin and forms soluble calcium chelates and removes the inorganic portion of smear layer.(1)

In ETDA/NaOCl protocol the specimens were first irrigated with 5ml of 17% EDTA followed by 3% NaOCl and finally rinsed with 5ml of distilled water. In this group AH Plus shows higher mean sealer penetration than Apexit and MTA Fillapex. EDTA/NaOCl protocol shows higher mean sealer penetration than

NaOCl/ETDA protocol but there is no significant difference observed between these irrigation protocol in sealer penetration. The combination of EDTA and NaOCl at a 1:1 ratio increases the effect of the chelating agent , and using NaOCl as last solution following EDTA in the present study also yielded superior results in sealer penetration .The combination of these solutions increases the pH, producing an alkaline environment in which EDTA has higher affinity for calcium ions. Thus NaOCl increases action of EDTA in EDTA/NaOCl protocol.(9),(17),(18),

This protocol has capacity to remove the inorganic (by EDTA) and organic (by NaOCl) components of smear layer. This fact may also explain the present results, that is, the EDTA/NaOCl protocol caused further exposure of the dentinal tubules and probably allowed greater sealer penetration into them. (19)

This study results showed that alternating the use of NaOCl and EDTA with distilled water in between (NaOCl/EDTA and EDTA/NaOCl protocol) was more capable of preventing the orifices of tubules from being blocked by smear layer than using NaOCl or EDTA alone (EDTA/CHX , NaOCl/Saline protocol) .(17).(18)

Therefore, the effect of a final flush with NaOCl after EDTA irrigation on sealer penetration was a matter of interest. In this study, no significant differencesin the maximum depth of penetration were found between NaOCl/EDTA and EDTA/NaOCl, indicating that a final flush with 3 % NaOCl does not have an additional effect on sealer penetration.The reason for this result has been speculated that a low concentration (3.%) with NaOCl might have less effect on accelerating dentinal erosion. Even if NaOCl dissolved the exposed organic matrix, it might not affect the dentinal tubules that have already been opened by EDTA irrigation.(11)

In EDTA/CHX protocol the specimens were first irrigated with 5ml of 17% EDTA followed by 2% chlorhexidine and finally rinsed with 5ml of distilled water. In this protocol AH Plus shows higher mean sealer penetration than Apexit and MTA Fillapex.

Chlorhexidine lacks the tissue dissolving capabilities and its absence of proteolytic action, which makes the dentinal surface more hydrophilic. Furthermore, the organic component of the smear layer was not removed; this resulted in a surface covered by the organic components of smear layer, which may also have interfered in the sealer penetration. When CHX solution was used as final irrigation, it had no additional influence on the sealer penetration. It has been recommended that CHX be used after smear layer removal as a supplemental final irrigation step because of its residual antimicrobial activity (12),(19). All these factors leads to significantly less sealer penetration in EDTA/CHX protocol when compared to NaOCl/EDTA & EDTA/NaOCl protocols.

In NaOCl/Saline protocol the specimens were first irrigated with 5ml of 3% NaOCl followed by Normal saline and finally rinsed with 5ml of distilled water. In this group AH Plus shows higher mean sealer penetration than Apexit and MTA Fillapex. In this group NaOCl removes only organic part of smear layer which led to significantly least sealer penetration observed among all other experimental groups.(19)

The results of the present study showed that AH plus sealer has significantly higher penetration efficacy followed by Apexit and least penetration observed with MTA Fillapex irrespective of the irrigation protocol used. These results are in agreement with those of other studies (K.R.Sonu et al , Mohammed Abdul Khader et al).(15)(3),(20).

AH Plus, an epoxy resin amine sealer, shows more sealer penetration because the flow of AH Plus is significantly higher than other sealers tested. Balguerie et al evaluated that AH Plus scores the best for adaptation to root canal wall, extent of tubular penetration, and adaptation to the peritubular dentin. This might be explained by the capillary action of the dentinal tubules. Sealer may be drawn into the tubules by capillary action and not by hydraulic forces created during root canal filling. (1),(2),(21). On the other hand, AH Plus being chemically cured may allow for compensation of polymerization shrinkage and exhibits zero polymerization stress (15).

Apexit is calcium hydroxide based sealer which show less penetration than AH Plus. A study by Mohammed Abdul Khader et al. found that the flow of the epoxy resin-based sealer (AH Plus) increases with time compared to calcium hydroxide-based sealer (Apexit), which has maximum initial flow and there is no difference in flow with time. AH Plus is having a greater depth of penetration, this may be attributed to the slow setting of AH Plus. The fast setting behaviour of apexit making it less penetration in to dentinal tubules.(20)

From the present study results it is observed that MTAFillapex shows least penetration than AH Plus and Apexit.MTAFillapex is another currently available calcium silicate based root canal sealer. This sealer consists of salicylate resin, diluting resin, natural resin, bismuth oxide, nanoparticulatedsilica, and MTA. It was developed to utilize the good features of MTA; relatively high levels of biocompatibility, antimicrobial activity, and sealing ability were reported for this material.(21), (15) . MTA Fillapex has shown low penetration probably because of the high solubility, abscence of hydrophilic characteristics and also low adhesion capacity of tag-like structures because of apatite formation by MTA (8),(23).

The results of the present study showed that in all experimental groups the maximum depth of the sealer penetration was significantly better in the coronal thirds and middle thirds than in the apical thirds of root canals.These results are in agreement with those of other studies(Patel et al , Sen et al).This could possibly be because there are more dentinal tubules in the coronal area, and the diameters of the tubules in the coronal area are larger than those in the apical area. Primary dentinal tubules are irregular in direction and density; some areas are devoid of tubules. Also, cementum-like tissue can line the apical root canal wall, occluding any tubules.(2),(24),(15).

It is important to note that the obturation technique influences the percentage of sealer penetration into the root canal walls. Cold lateral compaction technique was used in this study. It was reported that the area of sealer coated root canal wall in the coronal area was significantly higher when lateral compaction was used than vertical condensation. Furthermore, there was significantly more sealer in the lateral canals obturated using lateral compaction.(1)

The observation of the interface sealer/dentin can be done with longitudinal or cross-sectional sections. The direction of the tubules is mostly perpendicular to the root canal wall. The chance to obtain a section of the tubule is even for both cutting directions. In most studies, longitudinal sections are made, especially when the coronal or middle part of the root canal is evaluated or when the location of the evaluation is not mentioned. However, for thin or curved roots, this could create problems in the apical root canal; therefore, present study used cross-sectional sections.(24)

This study yielded results that confirm the role of irrigation protocol in the interaction between sealers and root dentin. Because NaOCl and CHX are incapable of removing the smear layer, the adjunctive use of a chelating agent or acids is recommended. The most common irrigant used for this purpose is ethylenediaminetetraacetic acid (EDTA). EDTA is a biocompatible, artificial amino acid with a pH 7 that is used as a root canal irrigant. Despite having no antibacterial effect, it restrains the growth and finally kills microbes by chelating with metallic ions needed for growth of bacteria. EDTA at concentrations of 15 -17% eliminates calcium from dentine leaving an organic matrix with no lethal effect to periapical tissues. The dual-irrigation regime of NaOCl and EDTA has been used for removing the debris and smear layer, resulting in successful debridement and aided in enlarging narrow or obstructed root canals.(12),(17),(18),(19).

V. Conclusion

Within the conditions of this in vitro study, the following conclusions can be made:

1. Final rinse with 3% NaOCl, after a rinse with 17% EDTA influenced the maximum depth of the sealer penetration.
2. Alternating the use of NaOCl and EDTA with distilled water in between (NaOCl/EDTA and EDTA/NaOCl) was more capable of penetration root canal sealer in to dentinal tubules than using NaOCl or EDTA alone.
3. EDTA/CHX shows better sealer penetration than NaOCl / Saline.
4. Use of CHX and Saline did not favor the sealer's penetration.
5. AH Plus sealer show significantly more sealer penetration than Apexit and MTA Fillapex , Apexit show better sealer penetration than MTA Fillapex. MTA Fillapex shows least sealer penetration.
6. In all experimental groups, maximum depth of sealer penetration was better in the coronal thirds than in middle and apical thirds.

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Confocal Laser Scanning Microscope Images for sealer penetration in four final irrigation sequence

Figure: 1 CLSM Images of three sealers in NaOCl / EDTA Protocol Results

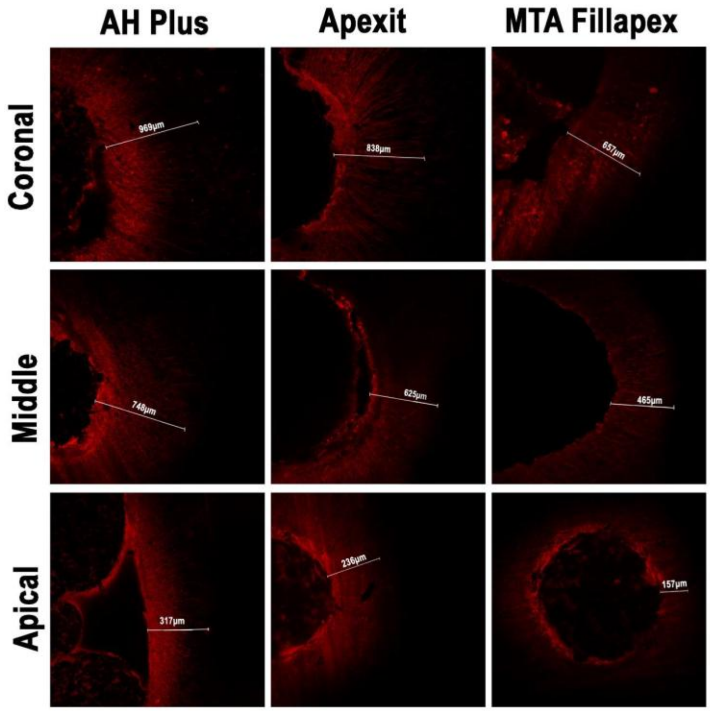


Figure : 2 CLSM Images of three sealers in EDTA/NaOCl Protocol Results

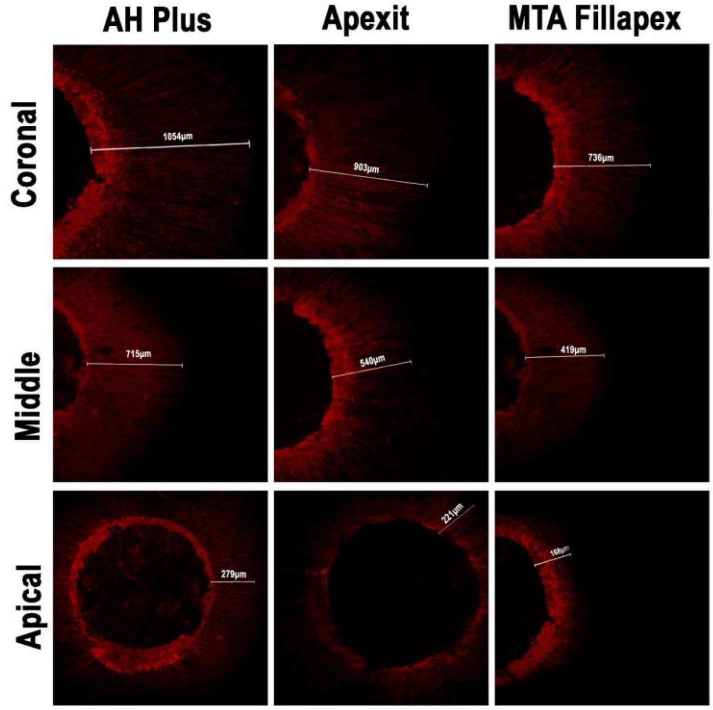


Figure : 3 CLSM Images of three sealers in EDTA / CHX Protocol Results

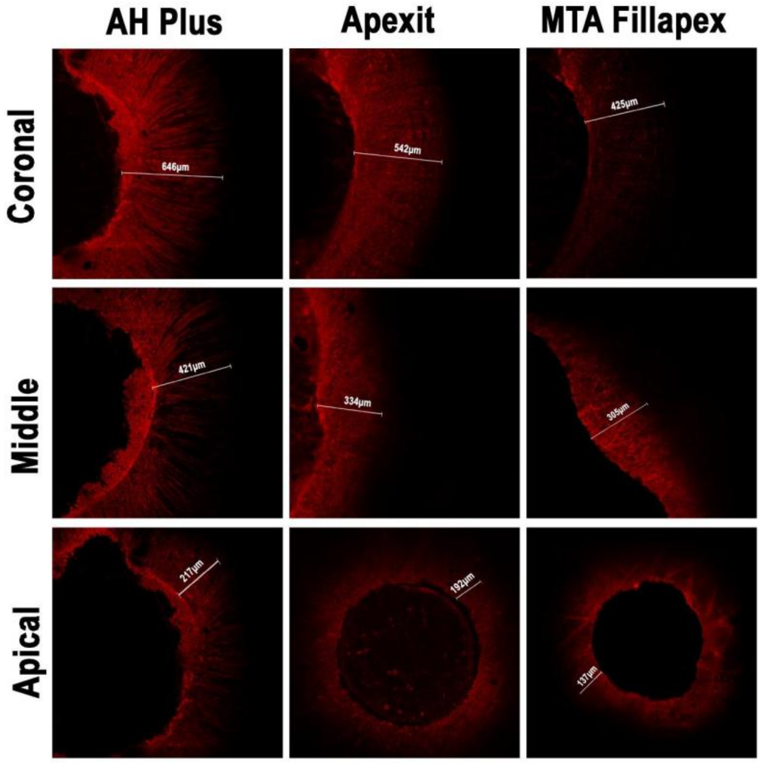


Figure : 4 CLSM Images of three sealers in NaOCl / Saline Protocol Results

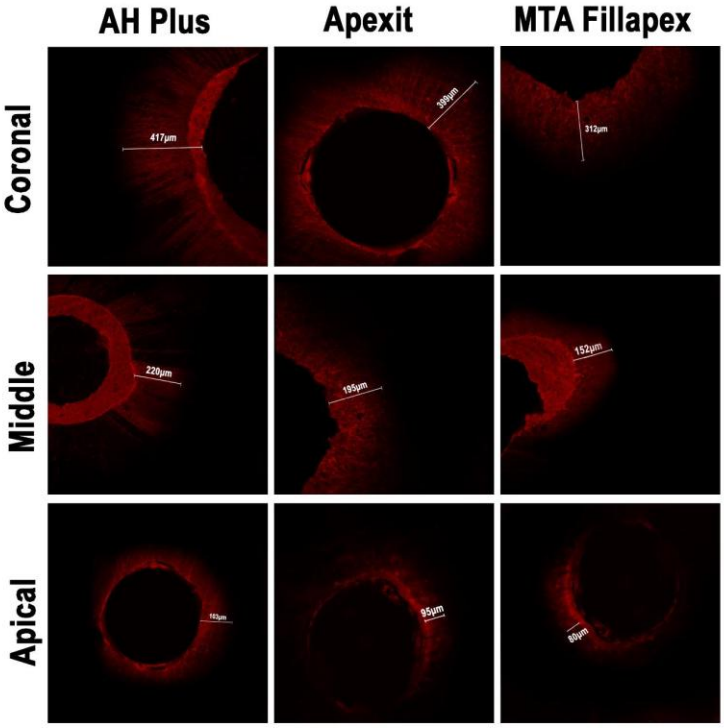


Table -1 shows the distribution of penetration of various root canal sealers indifferent parts of the root when canals were irrigated using different irrigation protocols.

Irrigation protocol		NaOCl+EDTA (GROUP- I)		EDTA+ NaOCl (GROUP- II)		EDTA+CHX (GROUP- III)		NaOCl +SALINE (GROUP- IV)	
Root third	**Sub Groups**	**Mean**	**S.D**	**Mean**	**S.D**	**Mean**	**S.D**	**Mean**	**S.D**
Coronal	AH plus (A)	969.00	32.128	1054.00	28.752	646.00	22.211	417.00	11.595
	Apexit (B)	838.00	100.642	903.00	103.928	542.00	18.738	399.00	19.120
	MTAFillapex (C)	657.00	28.304	736.00	69.314	425.00	12.693	312.00	13.984
Middle	AH plus (A)	748.00	33.928	715.00	81.001	421.00	11.005	220.00	10.541
	Apexit (B)	625.00	27.588	540.00	21.602	334.00	20.656	195.00	10.801
	MTAFillapex (C)	465.00	26.771	419.00	14.491	305.00	12.693	152.00	12.293
Apical	AH plus (A)	317.00	14.944	279.00	18.529	217.00	9.487	103.00	9.487
	Apexit (B)	236.00	27.162	221.00	13.703	192.00	13.166	95.00	9.718
	MTAFillapex (C)	157.00	14.944	168.00	102.935	137.00	14.944	80.00	8.165
Total	AH plus (A)	678.00	276.722	682.67	326.33	428.00	178.81	246.67	132.17
	Apexit (B)	566.33	260.523	554.67	289.58	356.00	147.19	229.67	129.36
	MTAFillapex (C)	426.33	210.754	441.00	246.41	289.00	120.84	181.33	99.26
	Total	556.89	268.812	559.44	302.76	357.67	159.67	219.22	123.00

Table-2 shows comparison of sealant penetration among various irrigation protocols (multiple comparison using Tukey's post hoc)

(I) Irrigation methods	(J) Irrigation methods	Mean Difference (I-J)	Std. Error	P value
hypo+edta	edta+hypo	-2.556	5.779	1.000
	edta+chlohex	199.222*	5.779	**.000**
	hypo+saline	337.667*	5.779	**.000**

edta+hypo	hypo+edta	2.556	5.779	1.000
	edta+chlohex	201.778*	5.779	**.000**
	hypo+saline	340.222*	5.779	**.000**
edta+chlohex	hypo+edta	-199.222*	5.779	**.000**
	edta+hypo	-201.778*	5.779	**.000**
	hypo+saline	138.444*	5.779	**.000**
hypo+saline	hypo+edta	-337.667*	5.779	**.000**
	edta+hypo	-340.222*	5.779	**.000**
	edta+chlohex	-138.444*	5.779	**.000**

Table -3 shows comparison of sealant penetration among root thirds (multiple comparison using Tukey's post hoc)

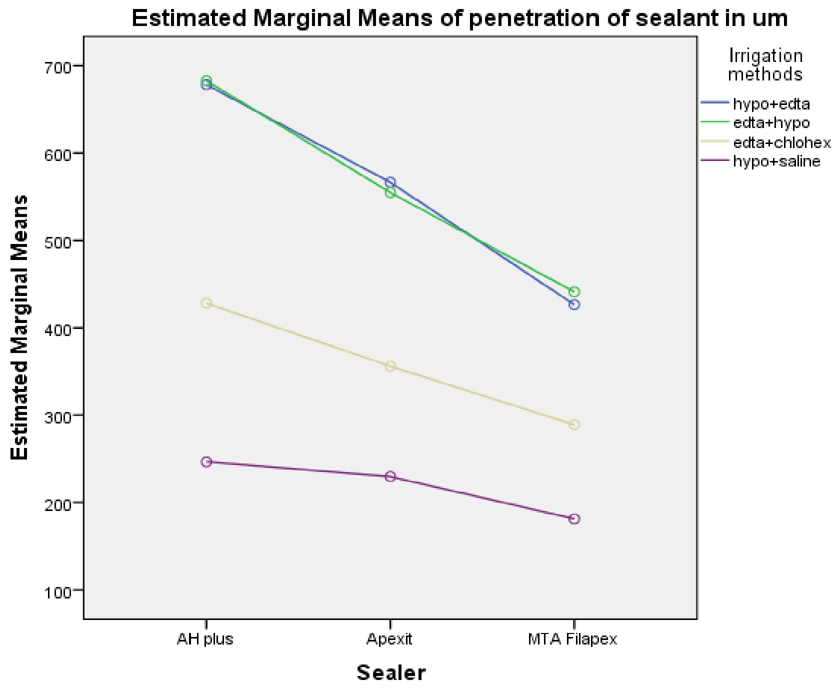
penetration of sealant						
(I) root third	(J) root third	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
coronal	Middle	229.917*	5.005	**.000**	217.873	241.960
	Apical	474.667*	5.005	**.000**	462.623	486.710
middle	coronal	-229.917*	5.005	**.000**	-241.960	-217.873
	Apical	244.750*	5.005	**.000**	232.707	256.793
Apical	Coronal	-474.667*	5.005	**.000**	-486.710	-462.623
	Middle	-244.750*	5.005	**.000**	-256.793	-232.707

Table -4 shows comparison of sealant penetration among 3 sealants (multiple comparison using Tukey's post hoc)

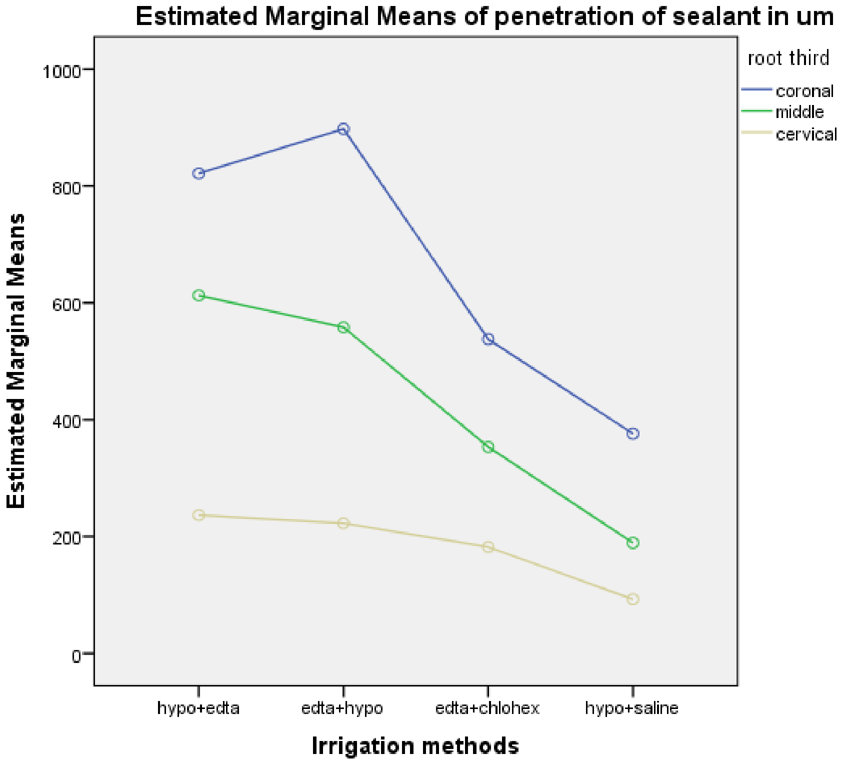
(I) Sealer	(J) Sealer	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
AH plus	Apexit	82.167*	5.005	**.000**	70.123	94.210
	MTA Filapex	174.417*	5.005	**.000**	162.373	186.460
Apexit	AH plus	-82.167*	5.005	**.000**	-94.210	-70.123
	MTA Filapex	92.250*	5.005	**.000**	80.207	104.293
MTA Filapex	AH plus	-174.417*	5.005	**.000**	-186.460	-162.373
	Apexit	-92.250*	5.005	**.000**	-104.293	-80.207

SEALANT PENETRATION MEANS PLOTS

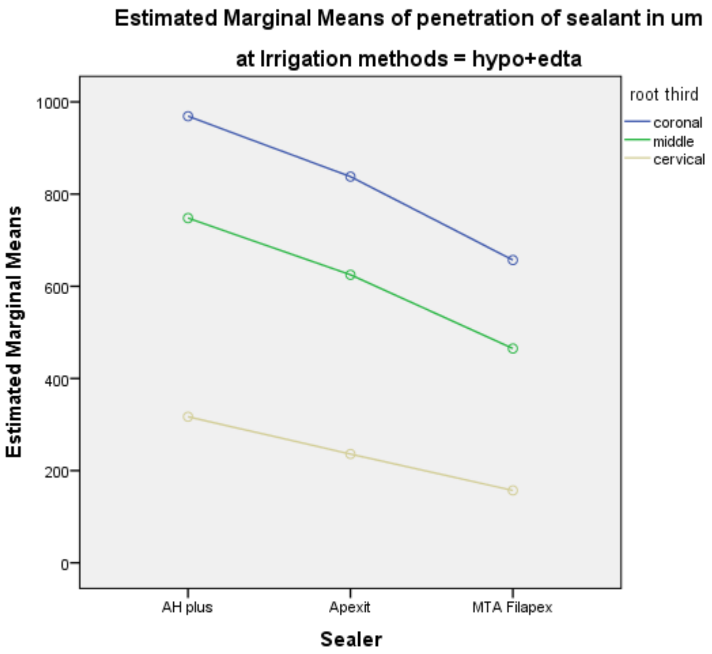
Graph – 1 shows Estimated Marginal Means of penetration of sealers in µm



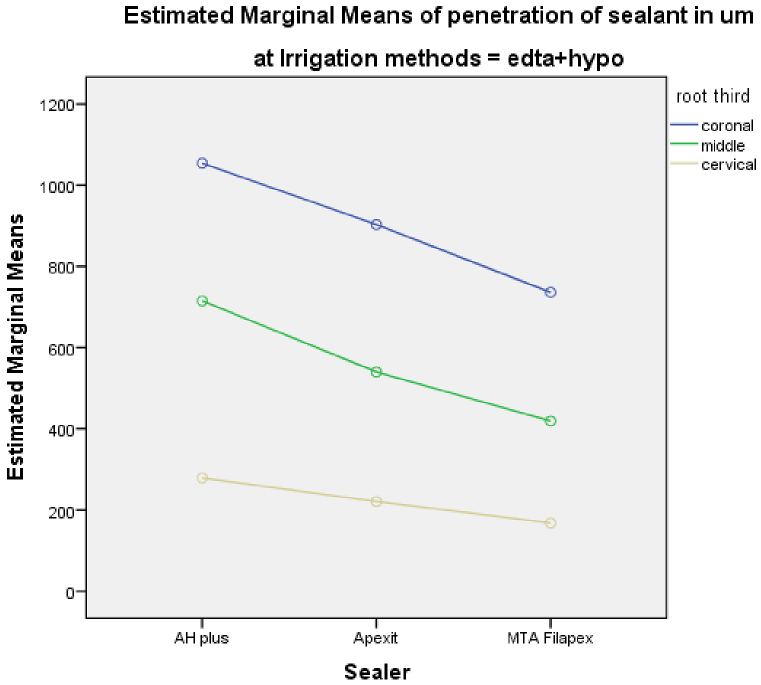
Graph – 2 shows Estimated Marginal Means of penetration of sealers in µm



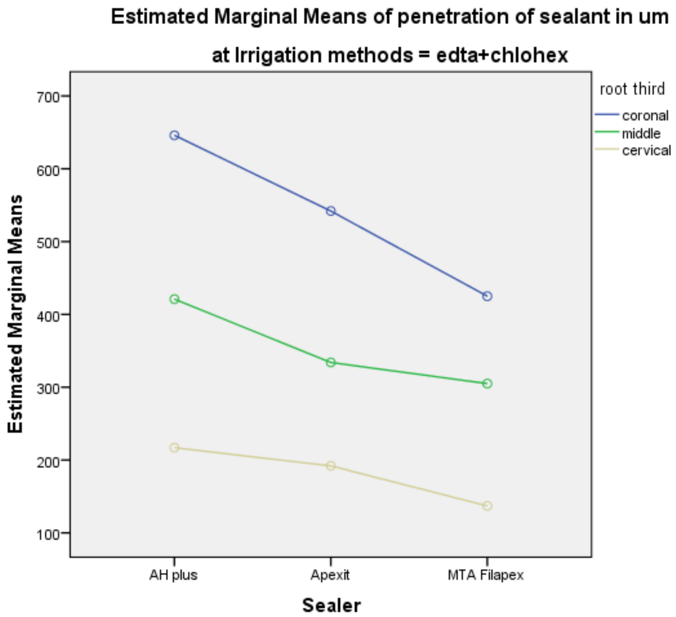
Graph – 3 shows estimated marginal means of penetration of sealers in µm at irrigation methods = NaOCl + EDTA



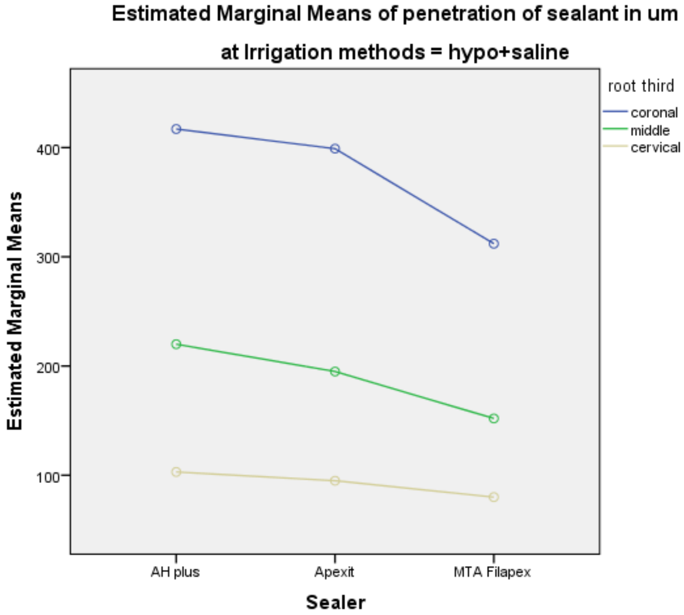
Graph – 4 shows estimated marginal means of penetration of sealers in µm at irrigation methods = EDTA+ NaOCl



Graph – 5 shows estimated marginal means of penetration of sealers in µm at irrigation methods = EDTA+ CHX



Graph – 6 shows estimated marginal means of penetration of sealers in µm at irrigation methods = NaOCl+ saline



. R.Rajasekhar Naik, et. al. "Influence of Different Irrigation Sequence on Penetration of Root Canal Sealers to Dentin- A Confocal Laser Scanning Microscopic Study." *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, 22(2), 2023, pp. 10-22.