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Evaluation of the push out bond strength on smear layer using three different root canal sealers - A comparative study

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Abstract

Background and objective: The key factor for any successful endodontic therapy, is having a good hermetic seal of a three dimensional obturation of the root canal. The formation of the smear layer after the instrumentation of the root canal plays an important role in the assessment of the bonding of root canal sealers and indirectly the push out bond strength

Purpose: To evaluate and compare the effect of smear layer affecting the push out bond strength of three different root canal sealers and the failure pattern in adhesion of calcium silicate, methacrylate and an epoxy resin based sealer in dentinal tubules of apical and middle portion of root canal using scanning electron microscope

Materials and Methods: Single-rooted mandibular premolars (n = 120) were prepared and divided into two groups (n = 60) based on irrigation regimen used:

GROUP 1: 0.9%SALINE and GROUP 2: 3%NaOCl + 17% EDTA. Further, according to the sealers used, each group was subdivided into 3 subgroups namely, SUBGROUP A (n=20) - Bioroot RCS, SUBGROUP B (n=20) – Hybrid Root SEAL and SUBGROUP C (n=20)- AH Plus sealer. After obturation with gutta-percha using three different sealer, roots were sectioned at 2 levels – apical and middle third of root canals and push-out bond strength test was assessed in the universal testing machine. One-way ANOVA with post hoc Tukey-hoc followed by Student Paired t Test significant difference tests were applied to assess the significance among various experimental groups. Samples of each groups were examined under SEM to determine the nature of the bond failures.

Results: The test results demonstrate that the Group 2C(AH Plus sealer without smearlayer) showed significantly higher mean Pushout Bond strength as compared to GROUPS 1B(Hybrid root SEAL with smearlayer), 1C(AH Plus sealer with smearlayer), 2B(Hybrid root SEAL without smearlayer)& 1A(Bioroot RCS with smearlayer) at P<0.001 & P=0.005 respectively. This was followed by GROUP 2A (Bioroot RCS without smearlayer) showing significantly higher mean pushout bond strength as compared to GROUPS 1B (Hybridroot SEAL with smearlayer), 1C (Ah Plus sealer with smearlayer) and 2B (Hybrid root SEAL without smearlayer) at P<0.001. Later, GROUP 1A (Bioroot RCS with smearlayer) showed significantly higher mean push out bond strength as compared to groups 1B(Hybrid root SEAL with smearlayer), 1C(AH Plus with smearlayer) and 2B(Hybridroot SEAL without smearlayer) at P<0.001. This in turn followed by GROUP 1C (AH Plus with smearlayer) showing significantly higher mean bond strength as compared to groups 1B(Hybridroot SEAL with Smear layer) & 2B(Hybridroot SEAL without smearlayer) at P<0.001 and finally GROUP 2B(Hybridroot SEAL without smearlayer) showed significantly higher mean push out bond strength as compared to GROUP 1B(Hybridroot SEAL with smearlayer) at P<0.001. However, no significant differences were noted between GROUP 1A(Bioroot RCS with smearlayer) & GROUP 2A(Bioroot RCS without smearlayer) [P=0.54].Between GROUP 2A(Bioroot RCS without smearlayer) & GROUP 2C(AH Plus without smearlayer) [P=0.36]

Conclusions: There was no statistical difference between pushout bond strength of Bioroot RCS. At the apical and middle third area of the root canal in absence of smear layer shows insignificant statistical difference between Bioroot RCS and AH Plus. Hybrid root SEAL without smearlayer showed significantly higher mean push out bond strength as compared when the smear layer is present.

Keywords: Adhesive and Cohesive, Ah Plus, Bioroot Rcs, Edta, Hybridroot Seal, Naocl, Pushout Bond Strength, Saline, Sem, Smear Layer

Introduction

One of the keys to successful root canal therapy is to adequately fill the prepared root canal space.[1]Root canal obturation provides a fluid tight seal to prevent the ingress of bacterial and their toxins and also their flow into the periapical tissue. As gutta percha obturating material does not seal the root canal system completely. The root canal sealers are used along with some surface alteration on the root surface.[2]

During any mechanical preparation either by hand or rotary there is production of an amorphous, granular, and irregular layer covering dentin, known as SMEAR LAYER. This layer consists of inorganic debris and organic components, such as pulp tissue remnants, odontoblastic processes, saliva, blood cells, and bacteria.[3]The SEM(Scanning electron microscopy) appreciate structure of smear layer.[4]Many researched have been tried using various irrigant namely, Sodium hypochlorite,EDTA ,the combination of irrigants and many more to remove the smear layer.

The use of a combination of EDTA (Ethylene -Diamine -Tetra-Acetic acid)and NaOCl (Sodium Hypochlorite) is commonly used for the effective removal of the smear layer from the root canal system.[5,6,7,8]

Several sealers & cements are available like zinc oxide eugenol cements,AH Plus,Diaket,Bioroot RCS,Hybrid root SEAL and many more.[9,10,11]

Zinc oxide eugenol sealers have a history of successful use over an extended period of time.

Zinc oxide eugenol sealers will absorb into the peri radicular tissues if extruded and they exhibit a slow setting time ,shrinkage on setting, solubility, and stain tooth structure.Antimicrobial property is the advantage of zinc oxide eugenol sealer.[11]

AH plus (Dentsply,Germany) proposed by Schroeder seemed to be possessing physicochemical properties because of its composition. Many studies using AH plus are have been to successful.[12]

Bioroot RCS (septodont),is a new calcium silicate based root canal sealer which is based on the mineralization potential of tricalcium silicate-based materials.

Although these modifications are aimed at improving the handling properties of the surrounding tissues and are aimed at avoiding tooth discoloration, they may influence the regeneration potential of the surrounding tissues.[13]

Hybrid RootSEAL(Sun Medical,Tokyo) The composition helps in acquiring the inherent properties of bonding.The major mechanism of bonding is achieve by formation of hybridized dentin which resist acidic challenges.[14]

Various tests used to measured bond strength are micro-tensile strength, shear strength testing and push out strength testing.[15]Among these tests push out bond strength test gives the measurement of interfacial shear strength between the different surfaces. The push out bond strength provides information about the adhesive property of the materials tested and helps to understand the resistance of the tested material to dislodgement meaning that material can bind to the tooth structure. Push out bond strength assessment is requires for root end filling perforation repair, obturation and the resistance to dislodgement of the root canal sealer material.Hence this study was under taken to compare and evaluate the effect of smear layer on the push out bond strength of three different recent root canal sealers.

One hundred twenty freshly extracted mandibular premolars were collected with single roots and then analyzed using digital radiograph to ensure that they had a single patent canal with root length were a minimum of 16mm(measured from the tip of the root to the cementoenamel junction.)and were then stored in normal saline solution at 4℃ until use. The working length was determined by using mangnifying loupe and calculated at 15mm and stored in normal saline at 4℃ .The sample were then dried and modelling or sticky wax was applied at the apical foramen. They were then placed in a transparent small plastic container into with a soft poly vinyl siloxane impression material had been placed.120 samples were then randomly divided into two experimental,namely Groups-GROUP 1 and GROUP 2 60 each samples.

The instrumentation was done first hand files upto size 15K followed by protaper universal rotary files from size Sx-F3. Irrigation was done by using 5ml of saline 0.9% for GROUP 1. 5ml of irrigant 3% NaOCl and 5ml of 17% EDTA used for GROUP 2 and were retained in the canal for 2 minutes and later dried using paper point. According to the sealer used, the samples in GROUP 1 and GROUP 2 were further divided into three subgroups, namely

SUBGROUP A (n=20) = Bioroot RCS

SUBGROUP B (n=20) = HybridRoot SEAL

SUBGROUP C (n=20) = AH Plus sealer

Obturation was completed by gutta percha along with the sealer following the single cone technique of size 30/0.06% (F3)

The samples which used Hybridroot SEAL as a sealer was cured for 20 seconds by using the light cure device in order to prevent the coronal leakage.Then the obturated samples was allowed to set for 1 week in a incubator of 100% humidity at 37°C.

Assessment of Pushout Bond Strength

All the obturated samples were then sectioned horizontally, perpendicular to the long axis and obtained a circular shape of the canal filling material at the thickness of 2mm. The thickness of each slice was measured using digital caliper (Insize Co. Ltd., Germany). Two slice from each root canal which was taken from the middle and apical third of the root canal were evaluated. The slices were stored in bottles filled with 1.5 ml distilled water for 2 days. Afterwards, each section was marked on its apical side and positioned on a base with a central hole in a universal testing machine. The materials dislocation resistance was measured using the push-out strength test with a universal testing machine (Instron, Model 5944 MicroTester Precision Instruments,Norwood, MA,USA)(IISC,BANGALORE) The push-out test was performed by applying a compressive load to the apical side of each slice using a cylindrical plunger attached to the upper portion of the testing machine with a crosshead speed of 1 mm/min. The load upon failure was recorded in Newtons (N) and divided by the bond area (mm2) to express the bond strength in megapascals (MPa).

Preparation of the sample for scanning electron microscopy analysis

After performing the push-out test, the fractured specimens were evaluated under a Scanning Electron Microscopy. Each of the specimens were sectioned in the bucco-lingual direction with the help of a safe-sided cutting disc under copious irrigation with distilled water using small cotton holder at the tip. The sectioned tooth sample which retained the obturation material was selected for observing under a scanning electron microscope (LEOVP435, Cambridge, UK). The sectioned parts were soaked in 15% EDTA solution for 10 minutes, followed by soaking in 3% NaOCl solution for 10 minutes, and then washed thoroughly with distilled water. Specimen were dehydrated and silver sputtered for SEM evaluation at the middle and apical thirds of the root canal. A SEM (NO. S-2400,Hitachi, Omeshi, Tokyo, Japan) was used at 1.3x magnification(CMTI ,BANGALORE). Each sample was categorized according to one of three failure modes: An adhesive failure that occurred at the dentin–material interface, cohesive failure that occurred within the material, or mixed failure, a combination of the two failure modes(Scoring failure mode according to Naga et al)Two investigators observed the adhesion failure on the surface of the root canal at middle and apical of each sample. SEM Photomicrographs were obtained using the digital analysis software.The most representative micrograph for each millimeter of sample is view in the middle and the apical third of the root canal.

Scanning electron microscope images of adhesion failure pattern

Figure 1: Group 1a:0.9%Saline (Bioroot Rcs)

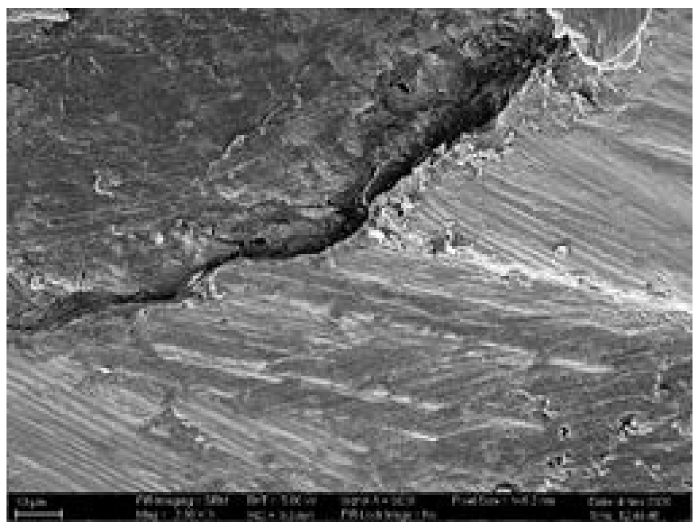


Figure 1 A: Middle third of the root canal

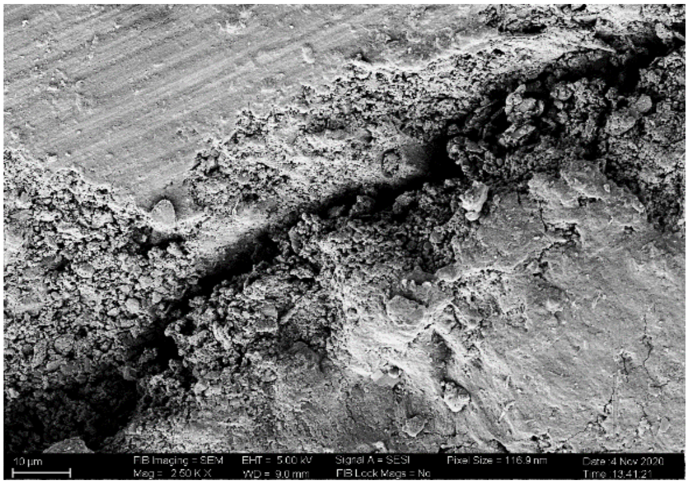


Figure 1 B: Apical Third of the Root Canal

Figure 2: Group 1B : 0.9% Saline Hybridroot Seal

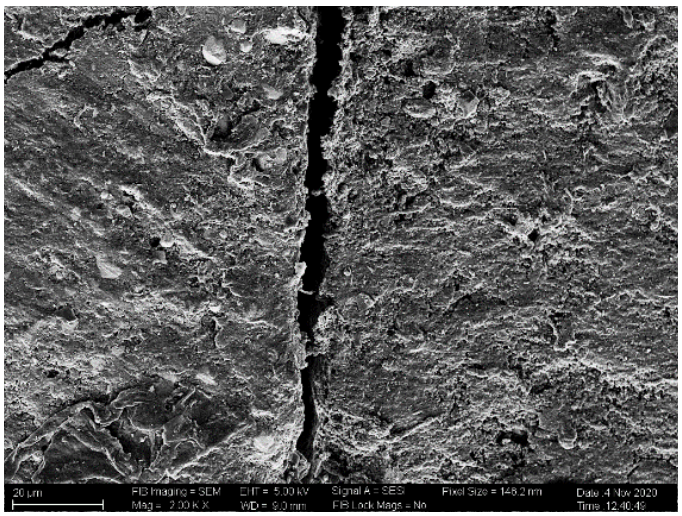


Figure 2 A: Middle Third of the Root Canal

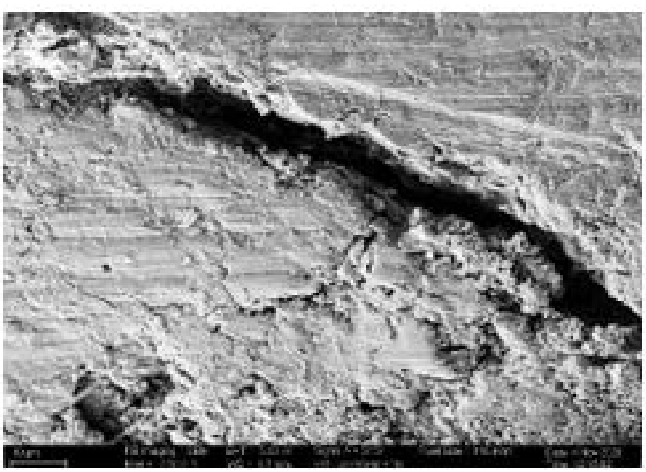


Figure 2 B: Apical Third of the Root Canal

Figure 3 group 1C: 0.9% saline (ah plus sealer)

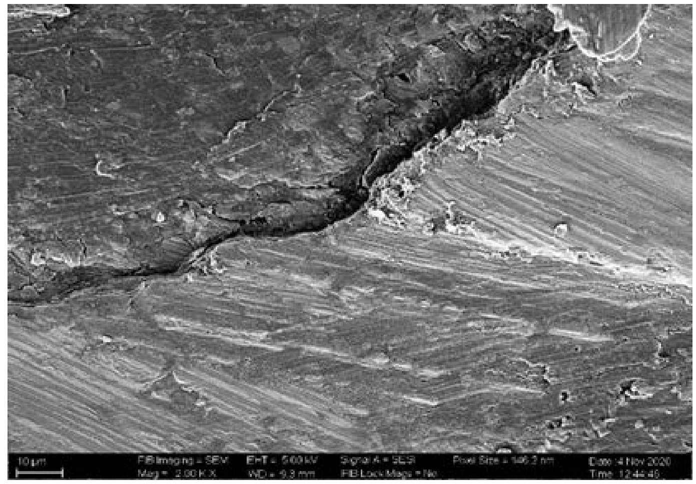


Figure 3 A: Middle Third of the Root Canal

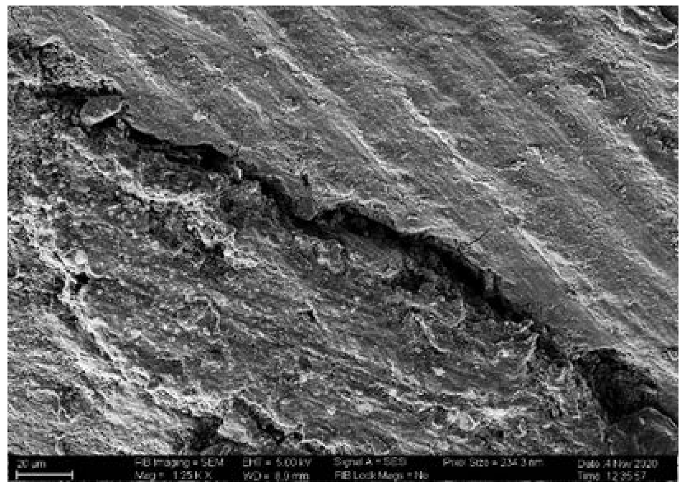


Figure 3 B: Apical Third of the Root Canal

Figure 4 Group 2A: 3%Naocl+ 17% Edta (Bioroot Rcs)

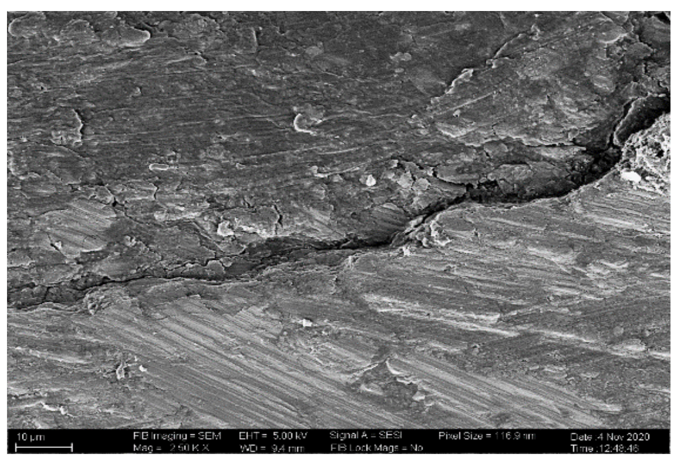


Figure 4 A: Middle Third of the Root Canal

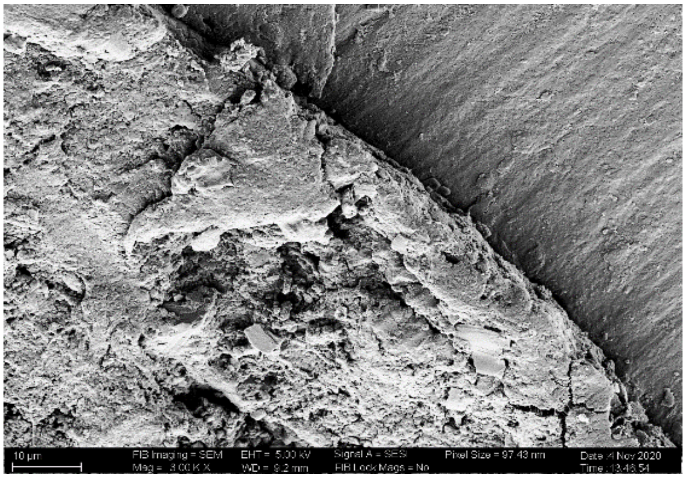


Figure 4 B: Apical Third of the Root Canal

Figure 5 - Group 2B: 3% Naocl + 17% Edta(Hybridroot Seal)

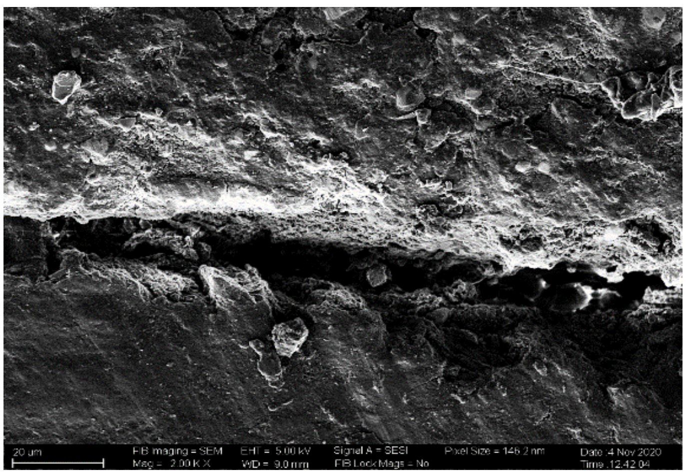


Figure 5 A: Middle Third of the Root Canal

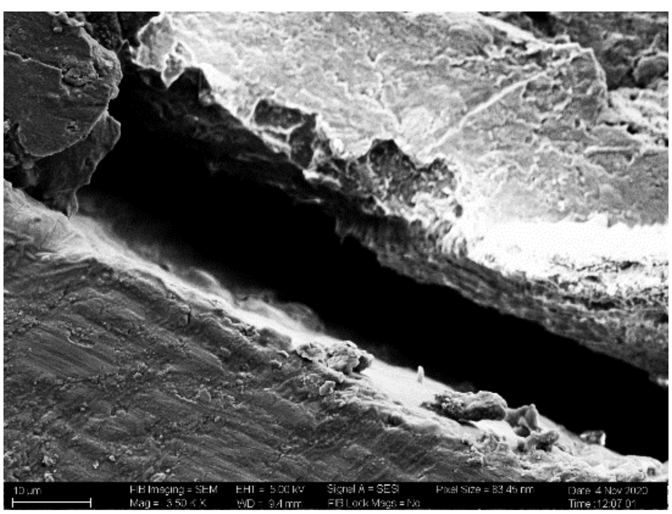


Figure 5 B: Apical Third of The Root Canal

Figure 6 Group 2C: 3%naocl + 17% EDTA (AH Plus SEALER)

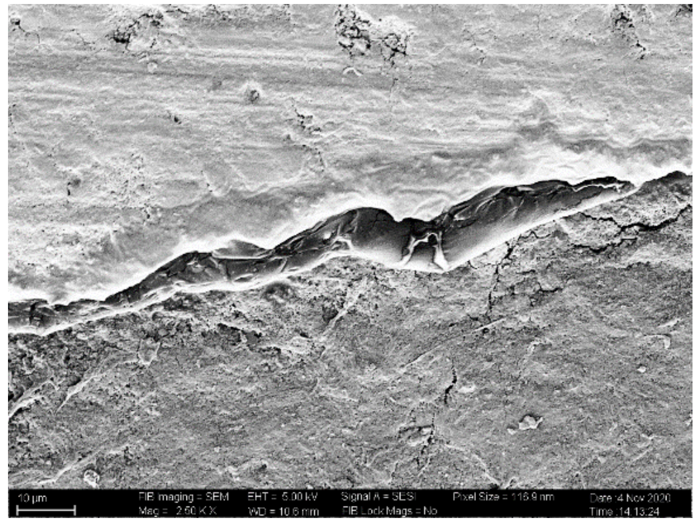


Figure 6 A: Middle Third of the Root Canal

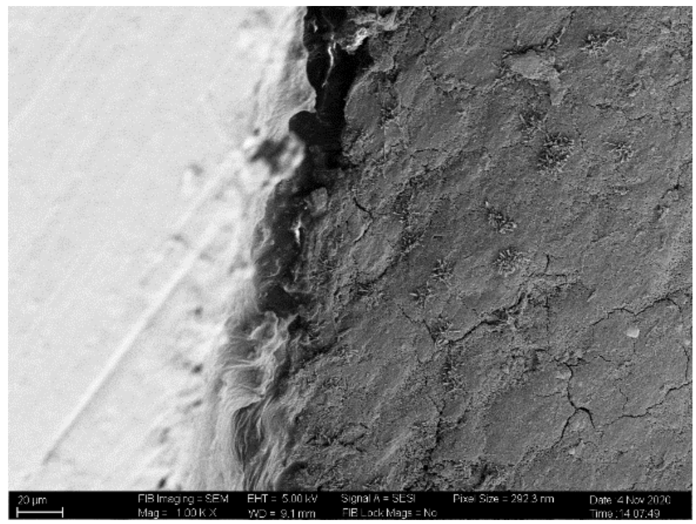


Figure 6 B: Apical Third of the Root Canal

Results

Inferential Statistics: One-way ANOVA test followed by Tukey's post hoc test was used to compare the mean Pushout bond strength between different study groups in Middle and Apical third regions.

Student Paired t Test was used to compare the mean Pushout bond strength between Middle and Apical third region in each study group.

The level of significance was set at $P<0.05$.

TABLE 1 : Comparison of mean Pushout Bond strength (in Mpa) in Middle third region between different study groups using One-way ANOVA Test

Table 1: Comparison of mean Pushout Bond strength (in Mpa) in Middle third region between different study groups using One-way ANOVA Test

Groups	N	Mean	SD	Min	Max	P-Value
Group 1A	20	7.228	0.803	5.89	8.74	<0.001*
Group 1B	20	3.006	0.519	2.07	3.97	
Group 1C	20	6.226	0.731	5.05	7.81	
Group 2A	20	7.607	0.824	6.24	8.89	
Group 2B	20	4.638	0.657	3.09	5.8	
Group 2C	20	8.051	0.673	6.83	8.86	

* - Statistically Significant

Table no. 1 compares the mean Pushout Bond strength (in Mpa) in Middle third region between different study groups.

The test results demonstrate that Group 1A showed mean Pushout Bond Strength values of 7.228 ± 0.803, Group 1B showed 3.006 ± 0.519, Group 1C showed 6.226 ± 0.731, Group 2A showed 7.607 ± 0.824, Group 2B showed 4.638 ± 0.657 and Group 2C showed a mean Pushout Bond Strength values of 8.051 ± 0.673. This difference in the mean Pushout Bond Strength values between different groups was statistically significant at P<0.001. [Refer Graph no. 1]

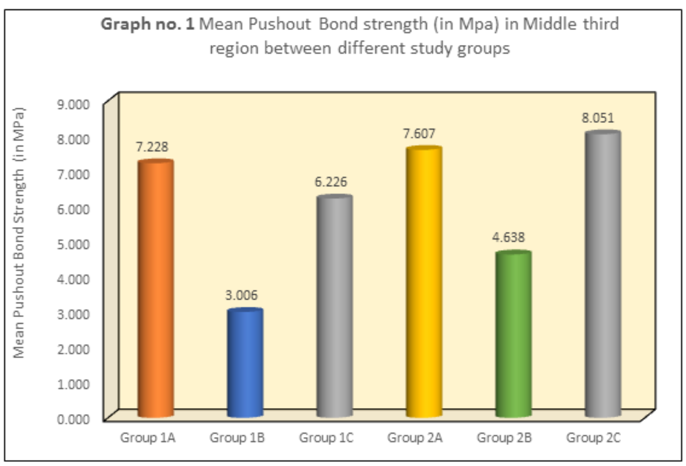


Table 2: Comparison of mean Pushout Bond strength (in Mpa) in apical third region between different study groups using One-way ANOVA Test

Table 2: Comparison of mean Pushout Bond strength (in Mpa) in Apical third region between different study groups using One-way ANOVA Test

Groups	N	Mean	SD	Min	Max	P-Value
Group 1A	20	6.158	0.418	5.07	6.78	<0.001*
Group 1B	20	2.443	0.334	2.08	3.08	
Group 1C	20	4.092	0.577	2.95	4.94	
Group 2A	20	6.431	0.569	5.43	7.45	
Group 2B	20	3.145	0.449	2.31	3.86	
Group 2C	20	6.746	0.650	5.83	7.84	

* - Statistically Significant

Table no. 2 compares the mean Pushout Bond strength (in Mpa) in apical third region between different study groups.

The test results demonstrate that Group 1A showed mean Pushout Bond Strength values of 6.158 ± 0.418, Group 1B showed 2.443 ± 0.334, Group 1C showed 4.092 ± 0.577, Group 2A showed 6.431 ± 0.569, Group 2B showed 3.145 ± 0.449 and Group 2C showed a mean Pushout Bond Strength values of 6.746 ± 0.650. This difference in the mean Pushout Bond Strength values between different groups was statistically significant at P<0.001. **[Refer Graph no. 2]**

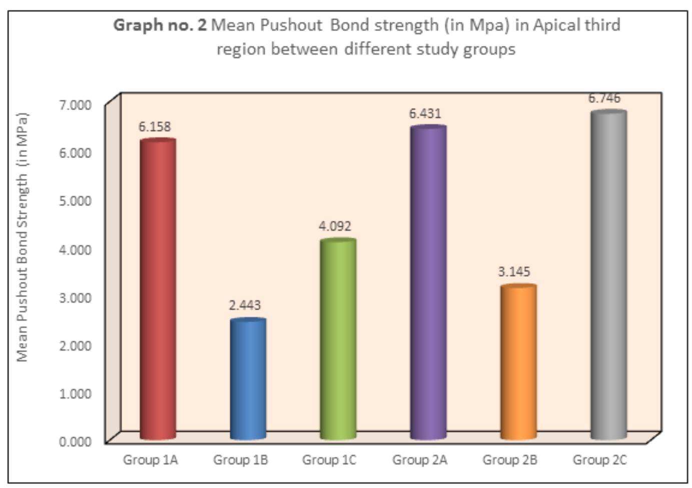


Table 3: Comparison of mean Pushout Bond strength (in Mpa) between Middle and Apical third region in each study group using Student Paired t Test

Table 3: Comparison of mean Pushout Bond strength (in Mpa) between Middle and Apical third region in each study group using Student Paired t Test						
Groups	Region	N	Mean	SD	Mean Diff	P-Value
Group 1A	Middle	20	7.228	0.803	1.070	<0.001*
	Apical	20	6.158	0.418		
Group 1B	Middle	20	3.006	0.519	0.563	0.001*
	Apical	20	2.443	0.334		
Group 1C	Middle	20	6.226	0.731	2.135	<0.001*
	Apical	20	4.092	0.577		
Group 2A	Middle	20	7.607	0.824	1.176	<0.001*
	Apical	20	6.431	0.569		
Group 2B	Middle	20	4.638	0.657	1.493	<0.001*
	Apical	20	3.145	0.449		
Group 2C	Middle	20	8.051	0.673	1.305	<0.001*
	Apical	20	6.746	0.650		

* - Statistically Significant

Table no. 3 compares the mean Pushout Bond strength (in Mpa) between Middle and Apical third region in each study group.

The test results demonstrate that the mean Pushout Bond strength in Middle third region was significantly higher [7.228 ± 0.803, 3.006 ± 0.519, 6.226 ± 0.731, 7.607 ± 0.824, 4.638 ± 0.657 and 8.051 ± 0.673] as compared to Apical third region [6.158 ± 0.418, 2.443 ± 0.334, 4.092 ± 0.577, 6.431 ± 0.569, 3.145 ± 0.449 and 6.746 ± 0.650] in each study group. This difference in the mean pushout bond strength between the middle and apical third region in all the groups was statistically significant at $P \leq 0.001$. **[Refer Graph no. 3]**

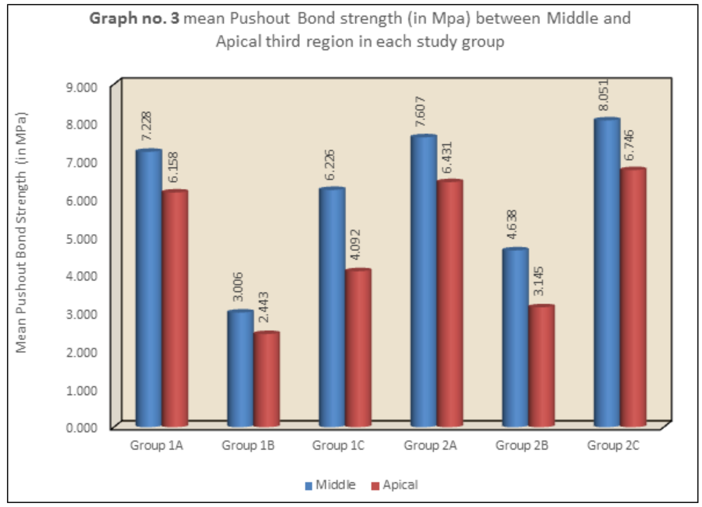


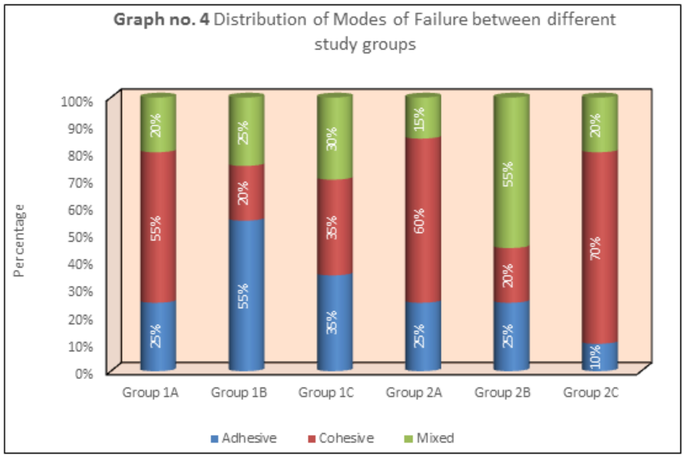
Table 4: Comparison of Modes of Failure between different study groups using Chi Square Test

Table 4: Comparison of Modes of Failure between different study groups using Chi Square Test							
	Adhesive		Cohesive		Mixed		
Groups	n	%	n	%	n	%	P-Value
Group 1A	5	25%	11	55%	4	20%	0.004*
Group 1B	11	55%	4	20%	5	25%	
Group 1C	7	35%	7	35%	6	30%	
Group 2A	5	25%	12	60%	3	15%	
Group 2B	5	25%	4	20%	11	55%	
Group 2C	2	10%	14	70%	4	20%	

* - Statistically Significant

Table no. 4 compares the Modes of Failure between different groups.

The test results demonstrate that the Adhesive failure was significantly higher in Group 1B [55%] as compared to Cohesive Failure seen in Group 1A [55%], Group 2A [60%], Group 2C [70%] and mixed type of Failure in Group 2B [55%]. However, no predominant type of failure was noticed in Group 1C. This difference in the modes of Failure between different study groups was statistically significant at P=0.004. [**Refer Graph no. 4**].



Discussion

The complete sealing and filling of the cleaned and shaped root canal system are important steps that can affect the long term success of root canal treatment .Because of the complexity of root canal system, sealers need to be used to fill the irregularities and to penetrate into dentinal tubules to obtain a hermetic seal of the root canal system. Meanwhile, root canal sealers should provide adherence between gutta-percha and dentinal walls to avoid gap occurrence at the sealer-dentine interface.[16] The main goal of obturation is to seal every lateral, furcal, accessory canals and the apex in the root canal system[17]The most accepted choice of the clinician in the obturation of the root canal is by using gutta percha material along with an appropriate sealer.As Guttapercha does not adapt to root canal walls, the use of sealers has been considered mandatory.It has been documented that teeth obturated with Gutta-percha along with sealer display a better seal than those obturated without sealer [18] Different types of sealers have been used in conjunction with Gutta-percha for root canal obturation with varied success.[19,20,21]

During mechanical preparation, the use of hand or rotary files for instrumentation will result in the production of considerable amount of mineralized debris consists of a layer of organic and inorganic materials what is called SMEAR LAYER. Eick et al.[22] were the first who identified the smear layer using scanning electron microscope (SEM) and found that smear layer is made from different size of particles of ranging from <0.5 to 15 µm. The presence of smear layer on instrumented root canals was first reported by McComb and Smith in 1975.[23] They showed that this layer is made of remnants of dentin, odontoblastic processes, necrotic or viable pulp tissues, and bacteria. Lester and Boyde[24]reported that smear layer is a mineralized collagen matrix made up of entrapment of organic matter within inorganic dentin. Other studies showed that the smear layer has an amorphous granular and irregular particle under SEM.[25] Smear layer removal prior to obturation of the pulp space still remains a controversial issue.

Researchers have reached to different conclusions on the importance of removing or leaving this layer. On one hand, it is a loosely adherent layer that can provide a pathway for microbial micro-leakage[26], it potentially harbors bacteria and can serve as a reservoir of irritants[27] , it can provide a substrate for any remaining bacteria following chemo-mechanical disinfection of the pulp space[28], and can prevent the penetration of irrigation solutions and inter-appointment medication into the dentinal tubules, thus jeopardizing the effective disinfection during root canal treatment, on the other hand, the smear layer can block the dentinal tubules and alter their permeability which can limit bacterial and toxin penetration[29]. Furthermore, bacteria surviving the disinfection protocol can be entombed within the dentinal tubules by the smear layer and the obturation material [30] It is very difficult to create a sterile environment in infected teeth after chemo mechanical preparation. The complexity of root canal systems leads to remains of pulp tissue and inorganic debris, mainly in the isthmuses or in areas that instruments did not reach. In this context, irrigants play an indispensable role. They must present with antibacterial activity and an ability to dissolve organic and inorganic tissues [31]

However, there is no single irrigating solution that alone efficiently covered all the functions required ,sometimes there is a combination of one or two irrigating solution in a specific sequence in order to obtained the goals of safe and effective irrigation.[32]

A large number of substances have been used as root canal irrigants, including acids (citric and phosphoric), chelating agent (ethylene diaminetetraacetic acid EDTA), proteolytic enzymes, alkaline solutions (sodium hypochlorite, sodium hydroxide, urea, and potassium hydroxide), oxidative agents (hydrogen peroxide and Gly-Oxide), local anesthetic solutions, and normal saline.

Normal saline is an isotonic solution to the body fluids and is being universally used as an irrigating material in all the surgical procedures including in the endodontic treatment.[33,34]

In isotonic concentration, though it does not produce any tissue damage it can flush out the debris from the root canal. Saline accomplishes gross debridement and lubrication. It should not be used alone as root canal irrigant, but as an adjunct to the chemical irrigant since saline helps in mechanical debridement.The main advantage of saline is that if it is inadvertently extruded into tooth periapical region, it does not produce any tissue damage. So, the chances of the acute inflammatory response are less.[35]

The most widely used endodontic irrigant is 0.5% to 6.0% sodium hypochlorite (NaOCl), because of its bactericidal activity and ability to dissolve vital and necrotic organic tissue. NaOCl solutions exert no effects on the removal of the inorganic components of the smear layer but it can be made possible by mixing the other chelating agent.[36]

EDTA (ethylene diaminetetraacetic acid) effectively dissolve inorganic material, including hydroxyapatite.[37,38,39,40] EDTA is most common used as a 17% neutralized solution (disodium EDTA, pH 7), but a few reports have indicated that solutions with lower concentrations (eg, 10%, 5%, and even 1%) remove the smear layer equally well after NaOCl irrigation. It removes smear layers in less than 1 minute if the fluid is able to reach the root canal wall surface. Goldberg and Spielberg (1982) have shown that the optimal working time of EDTA is 15 minutes, after which no more chelating action can be expected.[41]

By combining 5% sodium hypochlorite with EDTA, the bactericidal effect was considerably enhanced. Baumgartner and Mader found that alternating irrigation with NaOCl and EDTA was the most effective in removing both the smear layer and organic debris when using the ideal delivery of the irrigants.[42]

Root canal sealer help in the hermetic seal between the canal wall and core filling material is achieved by sealer which is critical for preventing root canal infection due to regrowth of microorganism or newly gained infection by apical or coronal leakage. The bacterial tight seal achieved by endodontic sealer is therefore a major aspect for assessing the properties of various endodontic sealer.[43]

Among the various root canal sealers available Bioroot RCS, Hybridroot SEAL and AH Plus sealer were taken for the study.

AH Plus sealer (Dentsply Maillefer, Switzerland) is an epoxy based endodontic sealer and presents with no photo polymerization system on its composition. It is believed that homogeneous polymerization occurs, leading to higher mean values of bond strength in the current study along the root canal. Chemical polymerization occurs at a low rate, delaying the gel point state and allowing for shrinkage stress relaxation, and avoiding a decrease in bond strength. This is in accordance with the study conducted by Wunderlich Rocha et al.[44]

In order to possess sealer which has high antimicrobial and low cytotoxicity property which promotes endodontic and periodontal regeneration recently available Bioroot RCS which has a prolonged release of calcium ions after setting seems to be advantages.[45]

Hybrid root seal is dual core and self etching sealer which has greater bond to the dentinal walls.[46]In order to enhance the function of the sealer producing better push out bond strength, this study was undertaken using the Bioroot RCS and hybrid root seal.

In this study the effect of chemical irrigants saline,sodium hypochlorite and EDTA were used to functioning on the presence and absence of smear layer.

Extracted single rooted human mandibular 1st premolars were taken for this current study in order to simulate the clinical situations.Standardization of the experimental groups in the present study was followed as the single rooted teeth with similar apical diameter fitting initial file and rounded canal cross-section were selected. In this study the root canals were prepared with a combination of the passive step-back technique and rotary nickel-titanium instruments using Protaper Universal system. This technique is an effective method to prepare root canals with rotary instruments. Rotary nickel titanium instruments (RNT) represent a relatively new approach to rapid and simplified canal preparation with a standardized uniform taper. In recent years RNT instruments with advanced blade designs have been developed to improve cleaning efficiency during root canal preparation. The Pro Taper file system has been one of the most frequently used and widely recommended RNT system. The Pro Taper cross sectional design resembles that of a reamer, with three machined cutting edges and convex core [47]. Hence the protaper universal is used for this study. A study conducted by Hengameh Ashraf et al, evaluated smear layer removal in the apical third of root canals by two chelating agents and laser prepared the apical region till size of 30/0.06 to allow adequate apical penetration of irrigants and access for the to the apical third of the canals.[48]

Injecting the irrigants by means of a syringe can control the volume and depth of syringe penetration and results in the flow of the solution to the apical third of the canal . So, all irrigation protocols in this study were done using 30 -gauge needles(close-ended single side vented) as it allows the clinician to place these as apical as clinically possible without canal binding amongst all the endodontic needles which is in accordance to study conducted by Gopikrishna et al.[49]

In this present study different types of irrigating solutions have been used .Based on the preserving or removal of the smear layer the samples were grouped into two groups, namely GROUP 1 and GROUP 2.

In Group 1 the study samples, were irrigated with 0.9% Saline to maintain the smear layer [50] In Group 2, the study samples,were irrigated with 2 ml of 3% of NaOCl and 17% EDTA to remove the smear layer. In both the groups the canals were irrigated respectively between each instrumentation using a 30 gauge needle according to Tuncer et al[51].

Studies done by Baumgartner et al on efficacy of several concentrations of sodium hypochlorite for root canal irrigation have shown that irrigation with 3 ml of NaOC1 after each instrumentation did an excellent job of removing superficial debris whether delivered with an endodontic irrigation needle or the ultrasonic device [52]

The same procedure was followed by rinsing of the canals with 5 ml of 0.9% saline to minimise potential interaction of NaOCl with any acidic irrigants that were employed as a final rinse. To prevent the escape of irrigants from the apex by simulating a clinical situation, the apex was sealed with aluminum foil coated with molten wax, simulating the clinical conditions.

This is in accordance to the method was followed by Hasnain et al[53]

The final irrigation in Group 2 was done by EDTA for 1 min in order to avoid the erosion of the dentinal tubules in apical third which is in accordance with procedure done by Doumani et al[54] in his study.

EDTA is normally used in a concentration of 17% and can remove the smear layers when in direct contact with the root canal wall for less than 1 minute according to Doumani et al .

Semra Çalt et al in the study on time-dependent effects of EDTA on dentin structures found that EDTA followed by NaOCl completely removed the smear layer in 1 min. In turn when EDTA is applied for 10 min,excessive erosive effects were observed with dissolution of peritubular

and intertubular dentin. According to the study findings, to inhibit the erosion on dentin, EDTA solution must not be applied for longer than 1 min[55]. Thus 5mL of 17% EDTA was used for 1 min in this study so that sufficient time is available for it to act in the apical third region and at same time, erosion of the dentinal tubules does not take place[56]. Also According to Saito et al[57]greater smear layer removal was found in the 1-min EDTA irrigation group. After the irrigation Group 1(0.9% Saline) and Group 2(3%NaOCl and 17%EDTA) each group were subdivided into according to the sealer used as, SUBGROUP A,SUBGROUP B and SUBGROUP C.

In SUBGROUP a Bioroot RCS root canal sealer was used. It is one of the recently introduced hydraulic tricalcium silicate-based sealer containing tricalcium silicate, zirconium oxide, etc. The release of calcium hydroxide from di- and tricalcium silicate cements due to hydrationand the contact with phosphate from tissue fluids leads to a precipitation of calcium phosphate or calcium carbonate on the material's surface[58,59]. Also, the formation of hydroxyapatite on a calcium silicate sealer, s surface after contact with phosphate has been reported [59]. This is the reason for the bioactive potential of tricalcium and dicalcium silicate sealers [60]. Furthermore, calcium silicates form an interfacial layer at the dentinal wall denoted as "mineral infiltration zone". The alkaline caustic effects of the calcium silicate cement,s hydration products degrade the collagenous component of the interfacial dentin [61]. This degradation leads to the formation of a porous structure that facilitates the permeation of high concentrations of Ca2+, OH−, and CO32− ions, leading to increased mineralization in this region[61,62]. This chemical interaction at the interfacial dentin along with a micromechanical interaction by tag-like structures is mainly the reason for measurable adhesion between calcium silicate-based materials and dentin[61,63]. In SUBGROUP B-Hybrid Root SEAL which is a dual-cured and a self-etching sealer, which does not require any additional priming or acid etching to the root canal dentin.

It has the advantage of forming a hybrid layer that creates a bond to the dentinal walls as well as the Resilon and gutta-percha.This material 4-META(4-methacryloxyethyl trimellitate anhydride) which is found in the liquid is able to promote monomer diffusion into the acid-conditioned and underlying intact dentin and produces functional hybridized dentin with polymerization.[64,65] The formation of hybridized dentin is the major mechanism of bonding and also the high-quality hybridized dentin resists acidic challenges.[66]According to Van Landuyt et al.[67], the two carboxylic groups attached to the aromatic group produce acidification and demineralization of the surface, and also enhance wetting, factors that are essential to promote adhesion of the material to the surface.Chang et al.[68] concluded that adhesive materials containing 4-META are capable of increasing the adhesion significantly This is because of monomer impregnation in the collagen fiber network and encapsulation of hydroxyapatite crystals. The demineralization of the superficial dentine matrix increases dentine porosity created by the dissolution of hydroxyapatite crystals within the collagen mesh, permitting the infiltration of adhesive system in the intertubular dentine, which probably favors hybrid layer formation. Gogos et al.[69] suggested the application of an adhesive system to the canal walls as a means to decrease the occurrence of leakage and increase the adhesion of the endodontic sealer by hybrid layer formation.

In SUBGROUP C, AH Plus sealer is an epoxy based endodontic sealer and presents with no photopolymerization system in its composition. It has been used in many studies due to its advantage of chemical polymerization and its effect on the bond strength it has been taken for the study. Thus AH Plus sealer was chosen for the present study. Obturation was done with single cone gutta percha in order to simulate the widely used method to maintain homogeneity among groups [70]

Different methods including micro tensile, shear bond, pull-out and push-out tests have been used for assessing the bond strength of dental materials to dentin. Among all these methods, micro tensile and push-out tests can be used to evaluate the bond strength in different parts of the root canal. But, preparing the samples for micro tensile test is very difficult and they may fracture before the test. On this basis, the pushout test is easy to perform without limitations as in that of micro tensile test with accurate and reliable results[71].Goracci et al. reported that thepush-out test is better which reflects the clinical conditions of the fracture pattern than micro shear or micro tensile methods, and is more reliable than other tests.[72]

Result in the present study shows, in Group 1 (0.9%saline) all the sealers in GROUP 1A (7.228), GROUP 1 B (3.006) and GROUP 1C (6.226) having lower bond strength.

The reason may be accounted to the sealers not able to penetrate the dentinal tubules, as the smear layer was intact. This is in accordance with the study conducted by V.Shivanna[73]. Bayram et al. in their study showed that removal of smear layer from the canal wall allows penetration of sealer into the dentinal tubules, thereby increasing adhesion to the root canal dentin.[74]

In Group 2 with combination of 3% NaOCl +17% EDTA it showed higher values with sealers (GROUP 2A=7.607, GROUP 2B=4.638,GROUP 2C=8.051) compared to values of Group 1 (0.9%saline).This is in accordance with the study that was conducted by Beltz et al., that sodium hypochlorite dissolves 90% of organic component of dentin and 17% EDTA dissolves 70% of inorganic components; the researchers suggested that using 10 ml of 17% EDTA for 1 min is the most effective method of smear layer removal.[75] Mohsen et al., suggested that 17% EDTA was more effective in the removal of smear layer from coronal and middle third as compared to the apical third.[76] Gharib et al.,[77] in a similar study, found that there were significantly less percentage and depth of penetration of sealer in apical sections than in the coronal and middle sections. Similarly, this current study also demonstrated more amount of sealer penetration in the middle(7.228,3.006,6.226,7.607,4.638,8.051) than the apical section(6.158,2.443,4.092,6.431,3.145,6.746). The physicochemical composition of endodontic sealers plays an important role in bond strength, tissue tolerance, and antimicrobial activity.[78,79] The clinical importance of sealer tags is to improve the adaptation as well as retention of the core material on the dentinal wall. The importance of smear layer removal has been investigated by number of authors [80]It has been suggested that the decreased microleakage associated with smear layer removal may be attributable to the deeper penetration of sealer into dentinal tubules. In the present study, SUBGROUP C (AH Plus sealer)have shown the better results when smear layer has been removed (GROUP 2C=8.051) and according to the result GROUP 2C had the highest push out bond strength compared to all the other subgroups(GROUP 1A=7.228,GROUP 1B=3.006,GROUP 1C=6.226,GROUP 2A=7.607,GROUP 2B=4.638) regardless of smear layer present/removed.

These values obtained for AH Plus sealer showed greater sealer penetration, which was due to the sealer integrity as well as the property of sealer being drawn into the tubules by capillary action. On the other hand, AH Plus being chemically cured may allow for compensation of polymerization shrinkage and exhibits zero polymerization stress. This study is in accordance with study conducted by Bouillaguet S et al.[81] and Iqbal et al[82].An impact of the final irrigation protocol on the push out bond strength has been reported for AH Plus [83]. The removal of the smear layer using EDTA after the use of NaOCl enforced the push out bond strength of AH Plus compared to other irrigation protocols[84] . The highest push out bond strength was found when NaOCl was used as final irrigant after the use of EDTA,compared to other irrigant combinations[85]

In this study it showed that GROUP 2-SUBGROUP C(8.051) showed higher push out bond strength than that of GROUP 2-SUBGROUP A(7.607).This is in agreement with the study conducted by David donnermeyer et al [86]that AH Plus has high resistance to dislodgement in general. The covalent bonds between the epoxy resin and the amino groups of the dentinal collagen [87,88] may result in a stronger link of AH Plus to dentin compared to the interaction of calcium silicates to dentin. The micromechanical interaction between the root canal wall and the calcium silicate based sealer (GROUP 2-SUBGROUP A=7.607) by the tag-like structures and the chemical interaction by the “mineral infiltration zone” 107establish a weaker link to the dentin compared to epoxy resins.(GROUP 2-SUBGROUP C=8.051)

In the present study, the use of the chelating agent EDTA as an irrigant significantly reduced the push out bond strength of BioRoot RCS which is in accordance to the study done by David et al[89].The reduction of calcium at the sealer–dentin interface or a degradation of the calcium silicate fraction in the sealer ,might hinder the formation of the “mineral infiltration zone” postulated by Atmeh et al.. This may result in a weaker interaction between the root canal wall and the sealer.

In the present study, SUBGROUP A(Bioroot RCS) showed better bond strength in GROUP 2 (GROUP 2-SUBGROUP A=7.607) compared to Group 1(GROUP 1-SUBGROUP A=7.228).

According to the results, the push-out bond strength was significantly affected by the sealer type and smear layer removal/preservation .Similar reasons were seen in the study conducted by Gutmann[90] the study reported that thermoplastic gutta-percha adapted well to canal wall after smear layer removal regardless of the presence of sealer. Studies conducted by Lester KS et al [91], Cergneux M et al[92],Foster KH et al [93] and Yang SE et al [94] found that Smear layer acts as a sealing barrier between the canal wall and root filling materialsand may compromise the ability to form a satisfactory seal. It could be also explained by the reason quoted by Shahravan et al [95] in his study a systematic review and meta-analysis which concluded that smear layer removal can promote an excellent fluid-tight seal, while other factors such as type of the sealer or the filling technique cannot produce significant effects .

The GROUP 1-SUBGROUP A(7.228)calcium silicate based sealer exhibited higher bond strength values compared to GROUP 1-SUBGROUP C (6.226)the resin based sealer. Possible reason for the result may be accounted to the study conducted by Atmeh AR et al,Jeong JW et al[96],Holland R et al[97],Gandolfi MG et al[98], Iacono F et al[99] and Weller RN et al[100] that the Calcium silicate produces a tag-like structure at the calcium silicate/dentin interface. The so-called “mineral infiltration zone” is a hybrid zone where hydroxyapatite recrystallization occurs when calcium silicate is applied in dentin . However, it has not been definitively proven that the mineral infiltration zone affects the outcome of endodontic treatment, positively or negatively. It might positively impact outcomes because calcium ions react with the carbon dioxide in the tissue to form calcite crystals .These crystals can reduce marginal gaps and porosity, and increase the retention of the cement.In their study they also proved, apatite deposition by a calcium silicate-based sealer did not reduce leakage because of its porous shape.

In the present study, SUBGROUP B (Hybrid root SEAL) shows less bond strength having smear layer preserved(SUBGROUP 1B =3.006) or removed(SUBGROUP 2 B=4.638) compared to the bio ceramic-based sealer (SUBGROUP A=Bioroot RCS) and epoxy resin based sealer(SUBGROUP C=AH Plus).

Accordance to Mai S et al[101] in their study it showed Hybrid Root SEAL being a methacrylate based sealers inherently undergo polymerization shrinkage coupled with high C-factor inside the root canals. Immediate light-curing from the coronal side of the roots may also create a large polymerization stress during setting by preventing flow of resin-based sealers and may lead to de-bonding of the resin from the root canal walls, which results in gap formation and subsequently affecting the sealing ability of the sealer. In the present study both middle and apical third the bond strength variations were observed. The values of the results was supported by the study conducted by Patel et al[102] who reported that mean maximum penetration in the cervical and middle third was greater than at the apical third.

Studies conducted by Gharib et al ,,Moon Y-M[103] et al and Kara TA et al[104] also reported decreased tubular penetration values in coronal areas as compared with apical thirds .Areas of sclerotic dentin are more common in the apical third [105]. In addition, the diameters of tubules in the apical third are smaller than those in the middle and coronal third, and the apical third has a lower number of tubules than the middle and coronal third[106].,also ,it is more diffcult to remove the smear layer from the apical third than middle and coronal third because of reduced irrigant delivery[107]. These factors might have influenced the findings of the present study. After the assessment of the push out bond strength of the sealers, their failure modes and the area is usually investigated for improvisation in the material science.Samples were categorized according to Nagas et al[108] as

ADHESIVE: (failure at the sealer dentin or the sealer-core material interface),

COHESIVE: (failure within sealer or dentin), or MIXED : (failure in both the sealer and dentin) .

Failure mode analysis revealed the different types of failures which were observed in all the different regions of the root canal system with respect to different irrigating solutions.

Several microscopy techniques are currently used to evaluate the sealer/dentin interface, including Stereomicroscopy, SEM (Scanning electron microscope), TEM (Transmission electron microscope) and CLSM(Confocal laser scanning microscope).[109]

An SEM was chosen for evaluating as it allow a highly descriptive and detailed observation of the dentinal tubules and the obturating material and the penetration depth could be calculated with greater accuracy throughout the sample sections.The results evaluated by SEM procedure showed that AH Plus predominantly displayed cohesive failure mode[FIGURE 3 and FIGURE 6] irrespective of the irrigation protocol which is in accordance to study done by David et al[89].BioRoot RCS mainly displayed mixed failure modes and adhesive failure was the second most common failure mode [FIGURE 1 and FIGURE 4].Similar result seen in study done by David et al[86].Hybridroot SEAL have showed cohesive and mixed bond failure pattern[FIGURE 2 and FIGURE4] which is in accordance to the study conducted by G.V Madhuri et al[110].Further studies are required to evaluate the effect of smear layer on the pushout bond strength of different root canal sealers.

Conclusion

- Within the limitation of this study:

• AH Plus sealer showed significantly higher bond strength compared to Bioroot sealer and

Hybridroot SEAL. The push-out bond strength of AH Plus was positively influenced by EDTA and NaOCl, had a negative effect on the BioRoot RCS; no influence in Hybridroot SEAL.

• Observation shows that smear layer removal is detrimental to the bond strength between calcium silicate cements and root canal dentin.

• Regarding the bond failure mode analysis there was no statistical difference between pushout bond strength of Bioroot RCS sealer with and without smear layer .Observation made at the apical and middle third area of the root canal in absence of smear layer shows insignificant statistical difference between Bioroot RCS and AH Plus.

• Hybrid root SEAL without smear layer showed significantly higher mean push out bond strength as compared when the smear layer is present.

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