EXPERIMENTAL COMPARATIVE EVALUATION OF THREE DIFFERENT DENTINE MODIFIERS ON PUSH-OUT BOND STRENGTH OF LUTED FIBER POST AND ITS RELATION TO RESIN TAGS FORMATION AS OBSERVED UNDER CONFOCAL MICROSCOPE
-AN INVITRO 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
05.	MA	Maleic 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.	HL	Hybrid Layer
13.	RT	Resin Tags
14.	OSHA	Occupational Safety and Health Administration
15.	CDC	Centre for Disease Control
16.	LED	Light Emitting Diode
17.	RVG	Radio Visio Graphy
18.	CEJ	Cemento-Enamel Junction
19.	WL	Working Length
20.	sec	Seconds
21.	SPSS	Statistical Package for the Social Sciences
22.	µm	Micro meter
23.	ANOVA	Analysis of Variance
24.	HSD	Honest Significant Difference
25.	SD	Standard Deviation
26.	S	Significant
27.	NS	Not Significant
28.	HS	Highly Significant
29.	N	Number of specimens

30.	p-value	Probability of obtaining a test statistic at least as extreme as the one that was actually observed
31.	Max.	Maximum
32.	Min.	Minimum
33.	No.	Number
34.	CI	Confidence Interval
35.	CHX	Chlorhexidine
36.	Bis-GMA	Bisphenol A Glycidil Methacrylate
37.	Mm	Milli Meter
38.	UTM	Universal Testing Machine
39.	AWL	Actual Working Length
40.	PUI	Passive Ultrasonic Irrigation
41.	ANP	Apical Negative Pressure
42.	CSI	Conventional Syringe Irrigation
43.	PIPS	Photon-induced photoacoustic streaming
44.	PSI	Post Space Irrigation

Introduction

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

- Henri Poincare

Long term prognosis of endodontically treated tooth is governed by placement of definitive post endodontic restoration that complements a successful endodontic treatment.[1] Post endodontic restorative treatment may vary from simple direct restorations to complex indirect restorations that may include placement of an intraradicular post and core.[2]

In contemporary endodontics, intraradicular posts and core have been primarily used to retain the coronal restoration in badly mutilated tooth.[3] Traditionally, metallic prefabricated and cast posts were utilized for decades.[4] However, with the advancement in the field of endodontics non –metallic posts have been introduced.

Fiber posts are generally, preferred in modern endodontics as they are biocompatible, resistant to corrosion and reduce the risk of tooth fracture.[5,6] Moreover, Fiber-reinforced posts have been routinely employed to restore endodontically treated teeth as their mechanical properties correspond to that of dentin. However, longevity of fiber post restoration depends on the effective bonding between the post, dentin and adhesive cement.[7]

Besides, adhesion between resin and dentine is regarded as a weak link and post cementation remains a clinical challenge as it is influenced by number of factors such as: polymerization shrinkage**,** poor control of moisture**,** disproportion between fiber post and diameter of post space.[8,9,10]

Naumann M et al. (2012) conducted a observational clinical study of endodontically treated teeth restored with adhesively-luted fiber posts and demonstrated an annual failure rate of **4.6%**.[11]

Furthermore, after post-space preparation, the dentinal wall is covered with smear layer which obliterates the dentinal tubules**,** thus reducing dentinal permeability which might prevent effective resin bonding.[12,13]

Previous research has suggested that the efficacy of the dentin adhesives mostly depends on the smear layer removal. Thus, smear layer removal plays a pivotal role in formation of homogeneous biomechanical unit and access the success of fiber post restoration.[14] However, removal of smear layer remains a troublesome procedure due to constriction and depth of post-space as well as, varied anatomy in apical region.

Researchers in their study have observed that Sodium hypochlorite (NaOCl) alone cannot remove the smear layer from the canal space and has to be used in adjunct to other chemical agents.[15]

Some researchers reported that these chemical agents cause alteration in the physical and mechanical properties of dentin and thus can be called as dentin modifiers. Use of various dentin modifiers has been suggested for removal of the smear layer that ultimately leads to increase in the bond strength of fiber post to root dentin. In particular, chelating agents are used due to their direct action on the calcium ions present in the hydroxyapatite crystal which is one of the main element in dentin.[16] Although EDTA effectively dissolves the inorganic material but has no or very little effect on the organic tissue.[17]

Kuah H et al. (2009) reported effective removal of smear layer after post space preparation using EDTA and NaOCl in adjunct with ultrasonic activation.[18] However, **Ballal N et al. (2009)** reported that EDTA removes the smear layer effectively only in Coronal and middle third of root canal.[19] Thus, owing to the limitations of endodontic irrigants, it is important to explore newer endodontic irrigants.

Several weak acids like maleic acid, citric acid and apple cider vinegar, have been reported as a potential irrigant in removal of smear layer form the canal space. **Wang L et al. (2017)** reported that maleic acid effectively removes the smear layer, even in the apical third of the canal with no adverse effect on the mechanical properties of root dentine.[20] **Fan F et al. (2017)** reported a significant improvement in bond strength of fiber post to root dentin after use of Maleic Acid.[21]

Also, as a growing trend to seek natural remedies, various plant and animal products have been tried and used in endodontic irrigation for removal of smear layer.

Chitosan is a naturally occurring polysaccharide biopolymer with an inherent antibacterial property. In recent years, use of Chitosan has become a significant area of research in endodontic irrigation due to its biocompatibility, biodegradability, bioadhesion and lack of toxicity. **Silva P et al. (2013)** studied the Smear layer removal capacity of Chitosan and stated that it effectively removes the smear layer from the canal space.[22] However, presently its effect on push out bond strength of fiber post to root dentin has not been evaluated.

Thus, this in vitro study was aimed to evaluate the bond strength of fibre post and its relation with the number of resin tags formation under Confocal Laser Scanning microscopy after treatment with three different modifiers, i.e 17% ethylenediaminetetraactetic acid (EDTA), 7%Maleic acid and 0.2%Chitosan before luting the fibre post.

The null hypothesis was that there is no significant difference on the push out bond strength of luted fibre post to root dentin when treated with 17% ethylenediaminetetraactetic acid (EDTA), 7% Maleic acid and 0.2% Chitosan and has no relation to the number of resin tag formation.

Aim & Objectives

Aim

To investigate the effect of 17% ethylenediaminetetraactetic acid (EDTA), 7% Maleic acid & 0.2% Chitosan on the push out bond strength of luted fibre post and its relation with resin tags formation at the resin-dentin interface under Confocal Laser Scanning Microscope.

Objectives

1. To evaluate push-out bond strength of a fibre post to dentin after use of 17% ethylenediaminetetraactetic acid (EDTA), 7% Maliec Acid and 0.2% Chitosan.
2. To compare push-out bond strength of fibre post to dentin after use of 17% ethylenediaminetetraactetic acid (EDTA), 7% Maleic acid and 0.2% Chitosan as modifiers.
3. To evaluate the resin tag formation after use of 17% ethylenediaminetetraactetic acid (EDTA), 7% Maleic Acid and 0.2% Chitosan under CLSM-imaging.
4. To compare the resin tag formation after use of 17% ethylenediaminetetraactetic acid (EDTA), 7% Maleic acid and 0.2% Chitosan as modifiers under CLSM-imaging.
5. To analyze the relationship between push-out bond strength and number of resin tag formation if any.

Review of Literature

Various surface treatment protocols and irrigation strategies have been tried in past for effective removal of smear layer from the post-space for effective bonding of post system with root dentin. Therefore, it is of vital importance to know these irrigants/ surface modifiers and techniques that various researchers have used and also difficulties encountered over the years for long term survival of post retained teeth.

Goldman LB et al. (1981)[23] in the SEM study evaluated the efficacy of 1% TEGO, REDTA, 5.25% NaOCl irrigating solutions. They reported that the smear layer was seen only in the areas with had been instrumented and could be removed by flushing the canal with 17% EDTA followed by 5.25% NaOCl. They also stated that if the smeared layer could be removed after preparation of the post space in the endodontically treated tooth, then the cement would enter the dentinal tubules to provide improved micromechanical retention.

Watson TF (1989)[24] conducted a Confocal Optical Microscope Study of the Morphology of the Tooth/Restoration Interface using Scotchbond 2 Dentin Adhesive, in an in vitro study with P50 composite resin used as the restorative material and observed that the Fluorescence imaging using Confocal microscope in which sections showed excellent adaptation of the adhesive to the tooth surface, with considerable penetration into the etched enamel structure without specimen damage when compared to conventional optical or scanning electron microscopy.

Czonstkowsky M et al. (1990)[25] observed that the motor-driven instruments, such as Gates-Glidden or post drills, produced quantitatively more smear layer than hand files.

Pashley DH et al. (1993)[26] investigated the comparison of the substructure of fractured dentin with that of smear layer-covered dentin, before and after acid etching, by high-resolution SEM. The results indicated that the most ideal dentinal substrate for bonding resins to dentin, with systems designed to infiltrate resin into the dentinal matrix, would be the demineralized dentin just beneath the surface of dentin that was acid etched and never air dried. However, the act of acid etching, at least with a solution of 37% phosphoric acid for 30 seconds, seemed to reduce the potential porosity of dentin, as revealed by the difference between the arrangement of collagen fibers at the surface and that beneath the surface, by creating a very thin surface film of condensed collagen fibers. This was even more exaggerated in dentin that had been covered by a smear layer prior to acid etching.

Mjor A et al. (2000)[27] studied the structure of dentin in the apical region of human teeth with emphasis on dentinal tubules and their branches. This descriptive histological study employed demineralized stained sections for light microscopy, demineralized unstained sections for scanning electron microscopy, and non demineralized acid-etched specimens for confocal tandem scanning microscopy. The sections showed marked variations in structure, including accessory root canals, areas of resorption and repaired resorptions, occasional attached, embedded and free pulp stones, varied amounts of irregular secondary dentin, and even cementum-like tissue lining the apical root canal wall. Also, the primary dentinal tubules were irregular in direction and density and some areas were devoid of tubules. They concluded that the irregular and variable structure of the apical region of human teeth represent special challenges during endodontic therapy. Adhesion techniques based on the penetration of adhesives into dentinal tubules are unlikely to be successful and adhesive techniques must depend on impregnation of a hybrid layer in apical region.

Mayhew JT et al. (2000)[28] studied the effect of root canal sealers and four different irrigation agents on retention of preformed posts luted with a resin cement. They found that 50% citric acid (C6H8O7) and 37% orthophosphoric acid (H3PO4) improved bond strength of resin cement to root canal dentin; whilst 5.25% NaOCl and 0.9% saline (NaCl) had no effect.

Calt S and Serper A (2002)[29] assessed the time dependent effects of EDTA on smear layer removal and structure of dentin after 1 and 10 min of application. Six extracted single-rooted teeth were instrumented to #60. Apical and coronal thirds of each root were removed, leaving a 5 mm middle third that was then cut longitudinally into two equal segments. Using 10 ml of 17% EDTA solution, halves belonging to the same root were irrigated for 1 and 10 min, respectively. All specimens were subjected to irrigation with 10 ml of 5% NaOCl after which they were prepared for SEM evaluation. The results showed that 1 min EDTA irrigation is effective in removing the smear layer. However a 10-min application of EDTA caused excessive peritubular and intertubular dentinal erosion. Therefore they suggested that irrigation with EDTA should not be prolonged for more than 1 min during endodontic treatment.

Serafino C et al. (2004)[30] evaluated surface cleanliness of root canal walls along post space after endodontic treatment using 2 different irrigant regimens, obturation techniques, and post space preparation for adhesive bonding. 40 teeth were divided into 4 groups, instrumented using Ni-Ti rotary files, irrigated with NaOCl or NaOCl + EDTA and obturated with cold lateral condensation or warm vertical condensation of gutta-percha. After post space preparation, etching, and washing procedure, canal walls were observed using a SEM. Amount of debris, smear layer, sealer/gutta-percha remnants, and visibility of open tubules were rated. Higher amounts of rough debris, large sealer/gutta-percha remnants, thick smear layer, and no visibility of tubule orifices were recorded in all the groups at apical level of post space. At middle and coronal levels areas of clean dentin, alternating with areas covered by thin smear layer, smaller debris, gutta-percha remnants, and orifices of tubules partially or totally occluded by plugs were frequently observed. They concluded that after endodontic treatment, obturation, and post space preparation, SEM analysis of canal walls along post space showed large areas covered by smear layer, debris, and sealer/gutta-percha remnants are not available for adhesive bonding and resin cementation of fiber posts. Hence it's necessary to develop other procedures to achieve a dentinal canal wall better prepared for adhesive resin cementation in endodontically treated teeth.

Bitter K et al. (2004)[31] investigated five different dental adhesives bonded to root canal dentin. Fifty extracted maxillary canines and central incisors were used. After root canal treatment the teeth were randomly divided into five groups of 10 teeth each. Fibre posts were inserted with five different adhesive systems and corresponding luting cements. Group 1:Clearfil Core/New Bond (Kuraray), Group 2:

Multilink (Vivadent), Group 3:Panavia 21/ED Primer (Kuraray), Group 4: PermaFlo DC (Ultradent), and Group 5: Variolink II/Excite DSC (Vivadent). The primer was labelled in each case with 0.1% Rhodamine B isothiocyanate (RITC). Each root was sectioned into 2 mm thick slices at 1, 4 and 7 mm below the cementoenamel junction. The resin–dentin interface was evaluated using a Confocal Laser Scanning Microscope; the thickness of the hybrid layer and the number of resin tags were measured. They found that conditioning of the root canal dentin with phosphoric acid and the use of one- and two- bottle-bonding systems gave a thicker and more uniform hybrid layer with considerably more resin tags than observed after the use of ‘self-etching’ adhesives. They also proposed that total etch systems might provide a more durable bond of the post to root canal dentin.

Goracci C et al. (2005)[32] evaluated the adhesion of fiber posts to intraradicular dentin. The interfacial strength and ultrastructure of a total-etch, self-etch and self adhesive resin cement used to lute endodontic glass fiber posts (FRC Postec, IvoclarVivadent) was assessed with the "thin-slice" push-out test and transmission electron microscopy (TEM). Interfacial strengths and microscopic findings indicated that the bonding potential of the total-etch resin cement was greater. The acidic-resin monomers responsible for substrate conditioning in self-etch and self-adhesive resin appeared unable to effectively remove the thick smear layer created on root dentin during post space preparation.

Garcia-Godoy F et al. (2005)[33] in their study comparing 17% EDTA and MTAD showed that 17% EDTA could effectively remove the smear layer from dentin of the root canal-treated teeth. They concluded that though both rinses effectively removed the smear layer, they could also cause a collapse of the dentine matrix structure which in turn would lead to poor resin infiltration and subsequent poor hybrid layer formation.

Kenshima S et al. (2006)[34] studied the conditioning effect on dentin, resin tags and hybrid layer of different acidity self-etch adhesives applied to thick and thin smear layer and observed that the etch-&-rinse adhesive presented the thickest hybrid layer and was the only adhesive to produce resin tags in high density and uniform distribution along the whole dentin surface, independently of the smear layer thickness.

Menezes MS et al. (2008)[35] evaluated the effect of composition of endodontic sealer and the time elapsed between root filling and post fixation on adhesion to root canal dentin. They concluded that the calcium hydroxide-based endodontic sealer (Sealer 26) did not influence the pattern of bonding to root dentin irrespective of depth and time evaluated whereas, the eugenol-based endodontic sealer (Endofill) had a negative influence on bonding in all regions of the canal when placed immediately following root filling. For the 7-day period, this negative influence was noted in the apical third only. The influence of canal depth, because of poor polymerization, was observed as the bond strength decreased from the cervical to apical third in all the groups. When posts are to be cemented immediately after canal filling, eugenol containing sealers are not preferred

Demiryürek EO et al. (2009)[36] studied the effect of different surface treatments on the push-out bond strength of fiber post to root canal dentin. Sixty extracted human maxillary incisor teeth were manually shaped with K-files using the step-back technique. ISO size 45 files were used as master apical files. Post spaces were prepared and then the root canals were subjected to one of the following 5 surface treatments: irrigation with 5% sodium hypochlorite (NaOCl); treatment with ethanol, ethyl acetate, and acetone-based cleansing agent (Sikko Tim); irrigation with 17% EDTA; etching with 37% orthophosphoric acid for 15 seconds; and etching with 10% citric acid for 15 seconds. Fiber posts were luted using self-etching/self-priming dual polymerized resin cement. From the coronal part of each root, 3 slides of 0.6-mm thickness were obtained. A push-out bond strength test was performed by a universal testing machine. Dentin surfaces were examined under scanning electron microscopy (SEM) after different surface treatments. They concluded that Sikko Tim group was the more effective surface treatment agent compared with EDTA, orthophosphoric acid, citric acid, and control groups; however, it could not remove the smear layer and sealer remnants effectively on radicular dentin surfaces. Removal of the smear layer and opening of dentinal tubules are not recommended when a self-etching/self-priming adhesive system is used.

Gu XH et al. (2009)[37] carried out an in-vitro study on 48 extracted sound anterior teeth to evaluate the effect of different irrigating solutions with or without ultrasonic activation on smear layer removal after post space preparation and the amount of dentinal tubule opening at the coronal, middle, and apical thirds of the root canal dentin surface. After post space preparation, teeth were assigned to six groups: Group 1, 14% EDTA; Group 2, 14% EDTA with ultrasonic activation; Group 3, 5.25% NaOCl; Group 4, 5.25% NaOCl with ultrasonic activation; Group 5, 0.9% NaCl; and Group 6, 0.9% NaCl with ultrasonic activation. Specimens were examined under a field-emission electron microscope and scored for debris removal and dentinal tubule opening at the coronal, middle, and apical thirds of the root canal. The results showed that 14% EDTA performed significantly better than 0.9% NaCl and 5.25%NaOCl in smear layer removal and dentinal tubule opening. They concluded that irrigation with EDTA without ultrasonic activation could effectively remove the smear layer and open dentinal tubules after post space preparation which is of great benefit to the bonding of fiber posts.

Ballal N et al. (2009)[19] carried out an in vitro scanning electron microscopic analysis. They evaluated the ability of 17% EDTA and 7% maleic acid in the removal of the smear layer from the human root canal system. Within the limitations of the study following conclusion was drawn: Final irrigation with 7% maleic acid is more efficient than 17%EDTA in the removal of smear layer from the apical third of the root canal system, which is a crucial area for disinfection.

Malyk Y et al. (2010)[38] carried out a cross sectional study on analysis of resin tags in root canal dentin. They evaluated the length, density and quality of resin tags formed by penetration of various types of adhesive systems into dentinal tubules at various cross sections of the root canal in correlation to the density of dentinal tubules. The confocal laser scanning microscopic images showed a lack of continuity of resin tag length, density and quality from cervical to apical region of root canal. Also, application of etch and rinse adhesive in contrast to the self etch adhesives provided formation of denser and more homogenous resin tags.

Scotti N et al. (2013)[39] studied the effectiveness of an active application of liquid etching, compared with the standard gel formulation on smear layer removal from post space walls and push-out bond strength of luted fibre posts. Human extracted teeth were collected and root filled. After post space preparation and cleaning with 10% ethylenediaminetetraacetic acid for 30 sec, teeth were assigned to four groups (n = 11) according to etching procedure: (i) 37% phosphoric acid (H3PO4) gel; (ii) 37% H3PO4 liquid applied with an endodontic needle; (iii) 37% H3PO4 liquid applied with an Endovac; (iv) no etching procedure (control group). Three teeth per group were sectioned longitudinally and prepared for SEM examination to evaluate the presence of smear layer, debris, sealer/gutta percha remnants, and the number of open tubules. Eight teeth per group were bonded with anetch-and rinse adhesive, and fibre posts were luted with aresin-based cement. After cutting, specimens were prepared for a push-out test. The result showed improve smear layer removal in Group 2, followed by Group 1, Group 3. They concluded that Liquid phosphoric acid applied with an endodontic needle yields better canal wall smear layer removal and higher bond strength values when an etch-and-rinse system was used.

Srirekha A et al. (2013)[40] evaluated the effect of different irrigating solutions with passive ultrasonic agitation on smear layer and debris removal after post space preparation in 60 extracted human mandibular premolars. The samples were randomly divided into four groups. Group A was treated with 10 % citric acid followed by passive ultrasonic irrigation. Group B was treated with 17 % EDTA followed by passive ultrasonic irrigation whereas Group C was conditioned with 36 % phosphoric without ultrasonic agitation. Group D (control) was treated with 3 % NaOCl and passive ultrasonic irrigation. Samples were sectioned and evaluated for debris and smear layer removal under SEM. They concluded that the coronal and middle third of the post space showed good smear layer and debris removal using citric acid and EDTA, along with ultrasonic agitation.

Sultan SE et al. (2013)[41] studied the effect of different surface treatments of luted fiber posts on push out bond strength to root dentin. 60 freshly extracted single rooted upper central incisor teeth were selected and decoronated. All root canals were instrumented, obturated, the post spaces were prepared to a depth of 11 mm. The specimens were divided into five groups according to the surface treatment performed to the post. Group 1:-no surface treatments (control group), Group 2:-surface treatment with chloroform, Group 3:-surface treatment as in group 2 in addition to the application of silane coupling agent (Calibra), Group 4:-surface treatment by sandblasting using 50 µm alumina particles, Group 5:-surface treatment as in group 4 in addition to the application of silane coupling agent (Calibra), A dual-polymerizing resin luting agent (Calibra) was used for cementation of posts. Three segments (1 mm each) from the cervical 1/3 of each root were obtained. Micro push out test was performed on a universal testing machine until bond failure occurred. Following results were drawn: Micro push out bond strength of the luting agent to the post was significantly affected by surface treatment and treating the surface of the post with airborne-particle abrasion followed by silanization resulted in the highest bond strength compared with other treatments.

Elnaghy AM (2014)[7] studied the effect of QMix irrigant on bond strength of glass fibre posts to root dentine. According to this study QMix is an effective irrigant that can remove smear layer, open dentinal tubules and simplify the irrigation protocol, without compromising the bonding strength of glass fibre posts cemented with a self-adhesive resin cement to root dentine.

Talebian R et al. (2014)[42] studied the effect of ascorbic acid on bond strength between the hydrogen peroxide-treated fiber posts and composite resin cores and concluded that ascorbic acid application increased the microtensile bond strength between the hydrogen peroxide treated fiber post and composite resin core.

Ekim S et al. (2015)[43] evaluate the efficiency of different irrigation activation techniques on smear layer removal. About 80 single-rooted human maxillary central teeth were decoronated. The samples were prepared by using ProTaper system to size F4 and divided into eight equal groups according to the final irrigation activation technique: Group I distilled water was used as an irrigant. Other groups were treated with 2.5% NaOCl and17% EDTA, respectively. Conventional syringe irrigation was used in (CSI, Group 2). Irrigation solutions were activated using passive ultrasonic irrigation (PUI, Group 3), EndoVac apical negative pressure (ANP, Group 4), diode laser (Group 5), Nd:YAG laser (Group 6), Er:YAG laser (Group 7), and Er:YAG laser using with photon-induced photoacoustic streaming (PIPSTM, Group 8). Teeth were split longitudinally and subjected to scanning electron microscope (SEM). Thus, following conclusions were drawn: Final irrigant activation with PUI, ANP, Nd:YAG, Er:YAG, and PIPS demonstrated to be effective in removing smear layer. ANP is the most efficient technique for removing smear layer in the apical part of the root canal system.

Ekambaram M (2015)[44] published a comprehensive review on bonding of adhesive resin to intraradicular dentin in which they concluded that: Several factors such as, presence of sclerotic dentin, very high cavity configuration or ‘c’ factor, inadequate visibility and access, difficulty in moisture control can pose great challenges in achieving optimum resin bonding to internal root dentin. The use of sodium hypochlorite during root canal treatment with 17% EDTA as a final rinse could be a suitable strategy in order to achieve optimum bonding with resin-based materials to internal root dentin The use of zinc-oxide eugenol-based root canal sealers could have a negative effect, whilst calcium hydroxide-based sealers may not have such an effect on bonding of resin- based materials to internal root dentin. Bonding of adhesive resins to internal root dentin with residual pulpal remnants on its surface can be severely compromised.

Giudice G et al. (2016)[45] in their in-vitro study analyzed the efficiency of different post space irrigation protocols in post space dentin cleaning. 28 single rooted teeth were endodontically treated. After post-space preparation samples were divided into 4 groups. In each group different irrigation protocols were performed as follows: EDTA (Group A), 37% orthophosphoric acid (Group B), EDTA + 37% orthophosphoric acid with ultrasounds activation (Group C) and the control group (Group D) the irrigation was not activated by ultrasounds. The SEM observation analysis showed that the smear layer presence decreased in the crown-apical direction. They concluded that the amount of debris remaining tends to increase from coronal to apical area. The protocols that used ultrasound activated EDTA alone or in association orthophosphoric acid are the most effective. The different dentin surface obtained with the various protocols is functional to the different methods of adhesion mandatory for post cementation. If the technique requires the use of a total-etch adhesive, the use of an association of activated irrigants that determine a smearless layer surface is preferred. When self-etch bonding is used, in which the adhesion interface is made by the smear layer, a less aggressive treatment of the post space is indicated.

Kul E et al. (2016)[46] studied the effect of alternative post space irrigation procedures on the cement strengths of posts attached with self-adhesive resin cement. The teeth were divided into 4 groups corresponding to the post space irrigation procedure and treated as: the distilled water group (control) received 15 mL of distilled water; the NaOCl+ethylenediaminetetraacetic acid (EDTA) group was treated with 5 mL of 5.25% NaOCl, 5 mL of 17% EDTA, and 5 mL of distilled water; the chlorhexidine (CHX) group was treated with 15 mL of 2% chlorhexidine solution; and the phosphoric acid (PA) group treatment consisted of etching the walls of the prepared post holes with 35% phosphoric acid. The results showed that EDTA in combination with NaOCl could be advantageous for post space irrigation when fiber posts are bonded with a self-adhesive resin cement.

Carvalho M et al. (2017)[47] studied the influence of endodontic irrigation protocols on bonding of adhesive systems to enamel and dentin. He concluded that endodontic irrigation protocols (5% sodium hypochlorite or 2% chlorhexidine gel + saline solution combined with 17% EDTA) do not jeopardize the bond strength of adhesive systems to enamel and dentin. He also suggested that endodontic irrigation protocols do not impair the bonding effectiveness of adhesive systems to enamel and dentin.

Wang L et al. (2017)[20] studied the effect of maleic acid on smear layer removal and mechanical properties of root canal dentin with respect to different time exposure.108 single canal premolars were instrumented with rotary-files and then randomly assigned to test groups receiving 7% MA for 30 s, 45 s, 1 min, or 3 min or to control groups treated with 0.9% saline or 17% ethylenediaminetetraacetic acid for 45 s. The micro-hardness, nano-hardness and elastic modules were measured before and after treatment, while the amount of smear and erosion in the coronal, middle and apical thirds in root canal were evaluated by SEM, finally, the fracture strength was assessed by vertical root fracture testing. They concluded that the cleaning efficacy and mechanical properties of root canal dentine varied with MA exposure time and application of MA for 45 sec is most promising for clinical use.

Fan F et al. (2017)[21] studied the effect of maleic acid on both the bond strength of fibre posts to root dentine and smear layer removal after post space preparation. 60 single-canal premolars were endodontically treated and randomly assigned in four groups: Group 1 [0.9% saline solution (control)]; Group 2 [2.5% sodium hypochlorite (NaOCl)]; Group 3 [17% ethylenediaminetetraacetic acid (EDTA) followed by 2.5% NaOCl]; and Group 4 [7% MA followed by 2.5% NaOCl]. Self-adhesive resin cement was used to test the adhesion of a glass-fibre post to the root dentine through a micropush-out test. SEM analysis was performed to examine and score the treated specimens for smear layer removal. They concluded that maleic acid has highest mean bond-strength values in the apical regions when compared to sodium hypochlorite and EDTA.

Jalali H et al. (2018)[48] studied the effect of different irrigants applied after post space preparation on push-out bond strength of a self-etch resin cement. 72 decoronated single-rooted premolars were cleaned, shaped, and obturated and incubated at 37°C and 100% humidity for seven days. Post space preparation was done to receive size 2 D.T. Light fiber post, and the specimens were randomly divided into six groups according to the post space irrigant used: normal saline (control group), 5.25% sodium hypochlorite (NaOCl)/15 seconds, 17% ethylenediaminetetraacetic acid (EDTA)/60 seconds, 2% chlorhexidine (CHX)/5 minutes, MTAD/5 minutes, and acid-etching/15 seconds. Final irrigation was done with normal saline and dried. Fiber posts were cemented using Panavia F2.0. After 24 hours, two mid-root slices of 1mm thickness were obtained from each specimen and were in a universal testing machine to measure push-out bond strength. They concluded that etching or irrigating the root canals with MTAD or EDTA after post space preparation increases the bond strength of Panavia F2.0 to dentin.

Alkhudhairy FI et al. (2018)[49] studied the effect of different irrigation solutions on the bond strength of cemented fiber posts. They compared the bond strength across 4 groups Group 1: 6.15% sodium hypochlorite; Group 2: 17% EDTA; Group 3: 6.15% NaOCl +17% EDTA; Group 4: 6.15% NaOCl + 0.12% chlorhexidine solution and concluded that EDTA irrigant solution produced higher bond strength and was more effective in removing smear layer than NaOCl.

Materials and Method

One hundred 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 mesio-buccal & mesio-lingual radiographs. The teeth were cleaned, disinfected and stored as per the recommendations and guidelines laid down by OSHA and CDC. (2003 report 17).[50] The selected teeth were stored in phosphate buffer saline solution (Severn, Biotech).[51]

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 root and 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) (PLATE-I)
- Explorer (GDC) (PLATE-I)
- Pair of Tweezers (GDC) (PLATE-I)
- Excavator (GDC) (PLATE-I)
- Hand Scaler (Satelec P5 Newtron Worktop Scaler, Satelec Acteon)
- Digital Vernier calliper (WorkZone Hand Tools) (PLATE-I)
- Cotton holder (GDC) (PLATE-I)
- Waste receiver (GDC) (PLATE-I)
- Mixing spatula (PLATE-II)
- Mixing pad (PLATE-II)
- Endodontic Microbrush (PLATE-II)
- Mini Endo Block (DENTSPLY) (PLATE-II)
- Straight hand piece (NSK) (PLATE-II)
- Double sided diamond disc (DFS) (PLATE-II)
- X-Smart Endomotor (DENTSPLY) (PLATE-III)
- Digital Radiovisiography System (Kodak 5100 RVG)
- Gates Glidden drills (MANI) (PLATE-III)

- Standard 2% K & H files # 10-80 (MANI) (PLATE-III)
- Reamers (MANI) (PLATE-IV)
- Peeso drills (MANI) (PLATE-IV)
- ProTaper Universal rotary files. (DENTSPLY) (PLATE-IV)
- Endodontic hand spreaders (MANI) (PLATE-IV)
- Endodontic hand pluggers (MANI) (PLATE-IV)
- Lentulospirals (MANI) (PLATE-IV)
- Hot shot tip (Discus Dental) (PLATE-V)
- LED Light curing gun (Bluephase-N, ivoclar, vivadent) (PLATE-V)
- L-moulds (PLATE-V)
- Precision cutting saw (IsoMet 5000, Buehler) (PLATE-XIV)
- Grinder & polisher (Buehler) (PLATE-XIV)
- Confocal laser scanning microscope (ZEISS with LSM Software ZEN 2007) (PLATE-XIV)
- Universal testing machine (ACME Engineers, Model no. UNITEST-10) (PLATE-XIV)

Materials:

- Root canal irrigation solutions (PLATE-VI)
  - Sodium hypochlorite (NaOCl) (Hyposept UPS Hygienes)
  - Normal saline (0.9 % w/v, Nirlife)

- 5ml syringe with 30 gauge needle (Nirlife) (PLATE-VI)
- RC Help (Prime Dental Products) (PLATE-VI)
- Paper points (DiaDent) (PLATE-VII)
- Gutta Percha points (DiaDent) (PLATE-VII)
- AH – Plus sealer (DENTSPLY) (PLATE-VII)
- Cavit (3M ESPE) (PLATE-VII)
- Auto polymerized clear Acrylic Resin(PLATE-VII)
- 17% ethylenediaminetetraactetic acid (EDTA) [Sybron Endo]. (PLATE-VIII)
- 7% Maleic acid. (Loba Chemie) (PLATE-VIII)
- 0.2% Chitosan. (Loba Chemie) (PLATE-VIII)
- Rhodamine B dye (Loba Chemie) (PLATE-VIII)
- Fibre posts (Angelus) (PLATE-IX)
- Prime and Bond NT (DENTSPLY) (PLATE-IX)
- Calibra resin cement (DENTSPLY) (PLATE-IX)
- Conditioner 36 (DENTSPLY) (PLATE-IX)

All the samples were radiographed with Kodak 5100 RVG system to eliminate the presence of any abnormality.

Sample Preparation

All teeth were decoronated 15±1 mm from the apex under copious water irrigation with a double sided diamond disc (DFS) 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 ProTaper Universal rotary files to size 40, 0.06 taper using the X-Smart-Endo-motor (Dentsply) in a crown down technique, under constant irrigation with 2.5% NaOCl using a 30 gauge needle.

The root canals then were dried with absorbent paper points and obturated by means of sectional obturation technique up to 5mm from the apex. AH plus (Dentsply) was used as a sealer in all the cases. The access cavities were temporarily filled with Cavit (3M ESPE) and stored at 37°C in 100% humidity for 7 days.

The root canals of each tooth were enlarged with peeso drills (Mani, Japan) using sizes 1-3 in ascending order. The depth of post space preparation was 10 mm for all the samples.

Distribution of Study Groups:

Following post space preparation the roots were randomly divided into four groups (n=25) corresponding to treatment with different irrigant solutions.

Groups	Sample Distribution	No. of Samples
Group I (G I)	Canal irrigated for 1 min using 5 ml of 0.9% normal saline solution	25
Group II (G II)	Canal irrigated for 1 min using 5 ml of 0.9% normal saline solution followed by 1 min irrigation with 5 ml 17% ethylenediaminetetraactetic acid (EDTA).	25
Group III (G III)	Canal irrigated for 1 min using 5 ml of 0.9% normal saline solution followed by 1 min irrigation with 5 ml 7% Maleic acid.	25
Group IV (G IV)	Canal irrigated for 1 min using 5 ml of 0.9% normal saline solution followed by 1 min irrigation with 5 ml 0.2% Chitosan.	25

Bonding of Fiber Post:

After post space irrigation, the samples in each group were thoroughly rinsed with distilled water. The root canal walls were then conditioned with 36% phosphoric acid (Dentsply) for 15 seconds, washed with water spray for 10 seconds and gently air dried. Excess water was removed using paper points. Equal drops of Prime and Bond adhesive and self cure activator were mixed, labeled with 0.1% Rhodamine B dye (Loba Chemie) and applied onto the root canal walls with a microbrush for 20 seconds. A single coat of Prime and Bond NT dual cure mixture was applied to the post. Both the canal and post were light cured for 10 seconds. Equal amounts of light and regular viscosity catalyst were mixed until uniform and applied into the root canal space with a lentulo drill. Then, the posts were inserted into the root canal, light cured for 20 seconds and excess cement was removed.

Sectioning of Tooth:

The excess fiber post was cut from the coronal portion of the root. All samples were embedded in cold-cure acrylic resin using L-moulds of dimensions 1.5 × 1.5 cm^2. Sections of the root were performed with a microtome precision saw (Isomet) at 1, 4 and 7 mm below the cemento-enamel junction (CEJ). Each section represented the coronal, middle and apical part of the post space preparation. The resulting sections of each tooth were (1±0.1mm thick). 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). The samples were kept humid during the whole study.

Methods of Measurements:

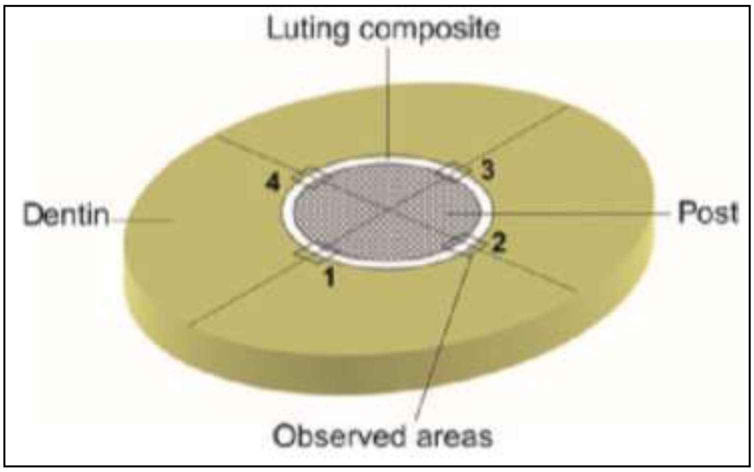
Two parameters were measured:

i. Number of tags formation into dentin.

ii. Push-out bond strength of luted fiber post.

Number of tags formation into 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.



Preparation of specimen. The measurements were taken at point 1-4 of the sample [31]

Images were recorded at four standardized areas of each sample. In order to quantify the thickness of the hybrid layer, the measurements were performed at four different locations on each image, and a mean was calculated. Thus, only one value (mean) per section entered the statistical analysis. The numbers of resin tags represented in the standardized images were counted and were scores as follows:[52]

The evaluation and scoring of resin tags were performed by two evaluators who were blinded about the irrigation protocol used to avoid odserver bias.

0	not detectable
1	few tags visible
2	uniform tags formation but with few lateral branches.
3	long resin tags with evident lateral branches.

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 post at a Crosshead speed: 1 mm/minute. The maximum failure load was recorded in Newtons (N) and Converted into megapascals (MPa) using following formula:

$$\textbf{Formula for Push out bond strength (MPa)} = \frac{\textbf{Push out Load (N)}}{\textbf{Area of bonded interface (sq/mm)}}$$

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

$\pi = 3.1416$, r = Radius of perforated cross section, h = Height of perforation

(Mean Area of samples: Coronal: $7.53mm^2$, Middle:$6.91mm^2$, Apical: $6.28mm^{2)}$

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

↓

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

↓

Obturation done using sectional obturation technique
upto 5mm from the apex

↓

The post spaces preparation (10±0.1 mm) using Peezo Reamer Drills

↓

Depending upon the type of dentin modifiers used, samples were divided
into 4 different groups (n=25)

Group I (Control)	Group II (EDTA)	Group III (Maleic Acid)	Group IV (Chitosan)

All the post were luted with adhesive resin luting system
and cemented into the post space

↓

Samples were mounted in acrylic block and sectioned at 1,4,7 mm from CEJ

↓

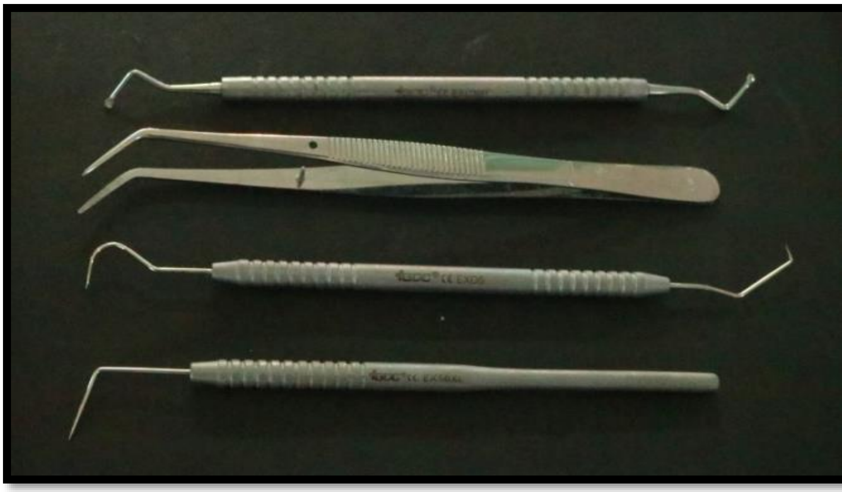
Confocal laser scanning microscopic analysis of no. resin tags

↓

Universal testing machine for push-out bond strength analysis

PLATE -I

ARMAMENTARIUM



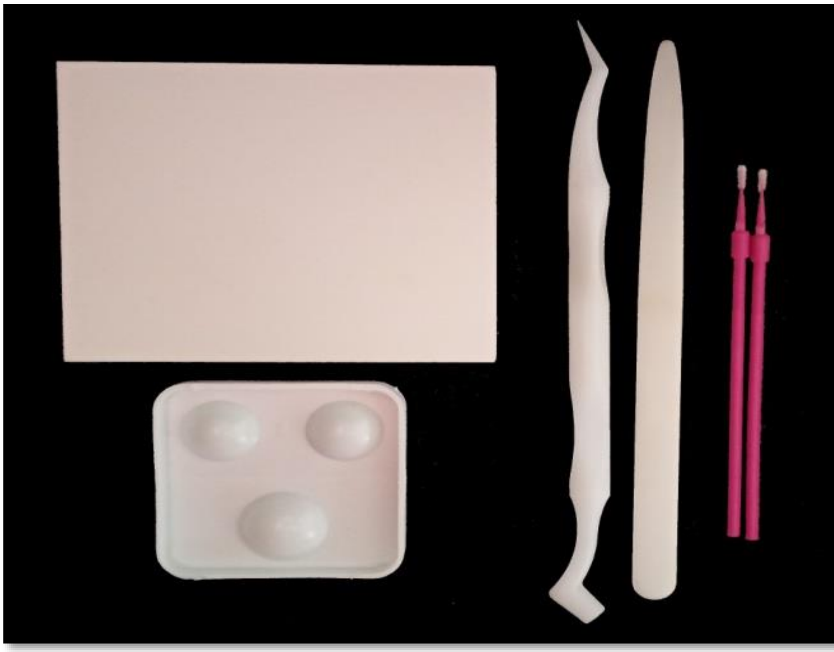
Hand Instruments (GDC)

Cotton Holder & Waste receiver (GDC)

Digital Vernier Caliper (Workzone tools)

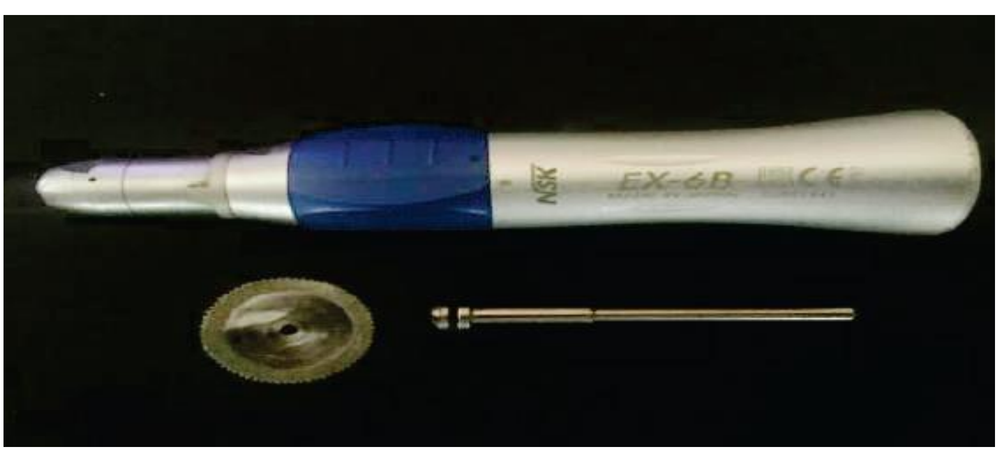
PLATE -II

ARMAMENTARIUM



Mixing pad, Spatula, Microbrushes, Dispenser

Endobloc (DENTSPLY)



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

ARMAMENTARIUM

Endodontic motor X-Smart
(DENTSPLY)

Gates Glidden Drills
(MANI)

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

PLATE -IV

ARMAMENTARIUM

Reamers (MANI)

Spreader & Pluggers (MANI)

ProTaper Universal Rotary Files (DENTSPLY)

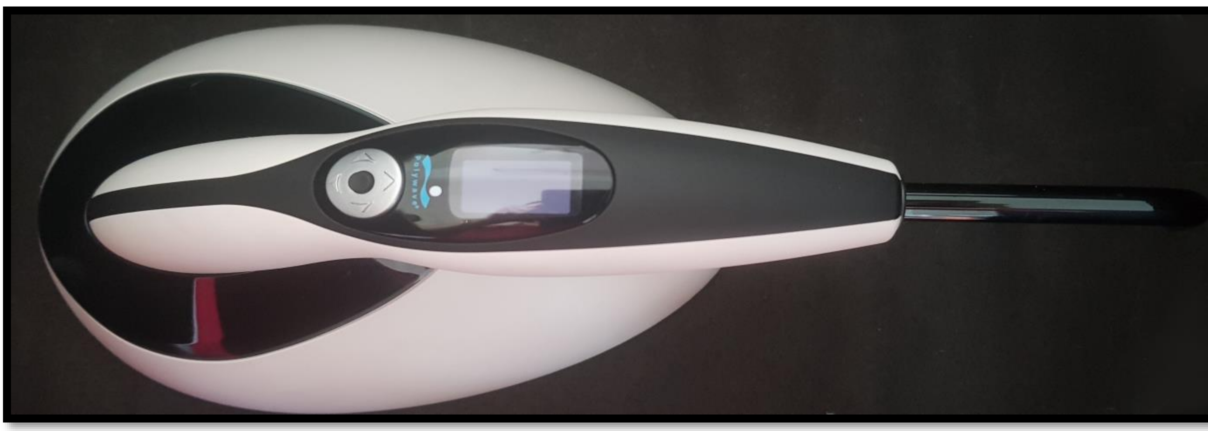
Peeso Drills (MANI)

LentuloSpiral (MANI)

PLATE -V

ARMAMENTARIUM

Hot tip (Discus Dental Inc.)



LED Light Curing Gun (Bluephase-N,ivoclar, vivadent)

L-Mould

PLATE -VI

MATERIALS

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

Irrigation Syringe , Side Venting Needle (Nirlife)

RC Help 17% EDTA (Prime Dental Products)

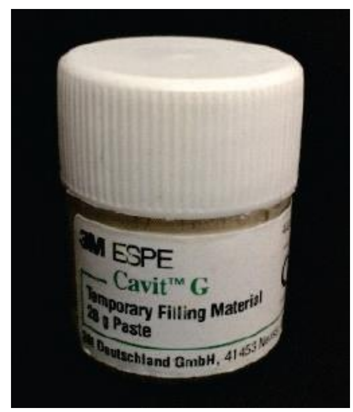
PLATE -VII

MATERIALS

Absorbant Points (DiaDent)

Gutta Percha Points (DiaDent)

AH Plus Resin Sealer (DENTSPLY)



Cavit G (3M ESPE)

Auto polymerized Acrylic Resin
(DPI-RR Cold Cure)

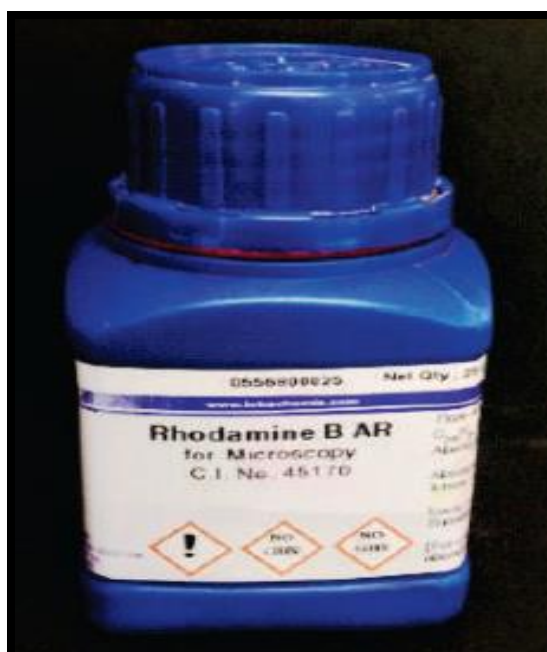
PLATE -VIII

MATERIALS

17% ethylenediaminetetraactetic acid (EDTA) [Sybron Endo]

7% Maleic Acid (Loba Chemie)

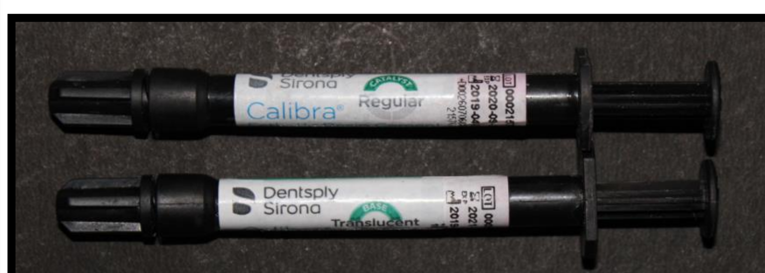
0.2% Chitosan (Loba Chemie)



Rhodamine B dye (Loba Chemie)

MATERIALS

Fibre Post (Angelus)

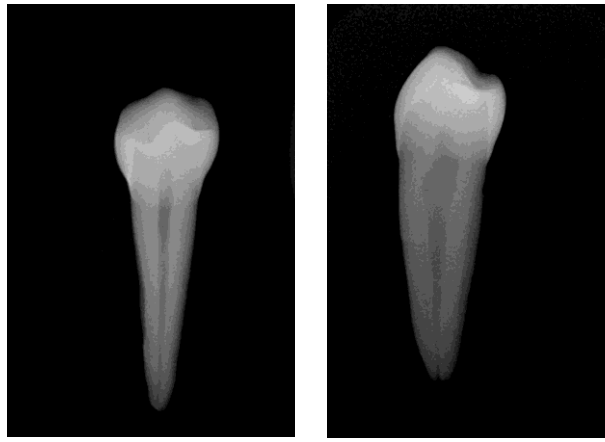


Prime and Bond NT, Calibra Resin Cement (DENTSPLY)

PLATE -X

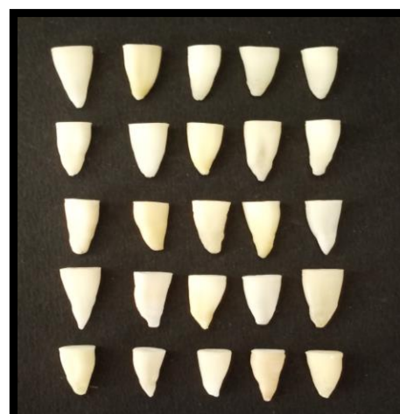
METHODOLOGY

Sample size (N=100)



Pre Operative Radiographs

Decoronation Of Samples

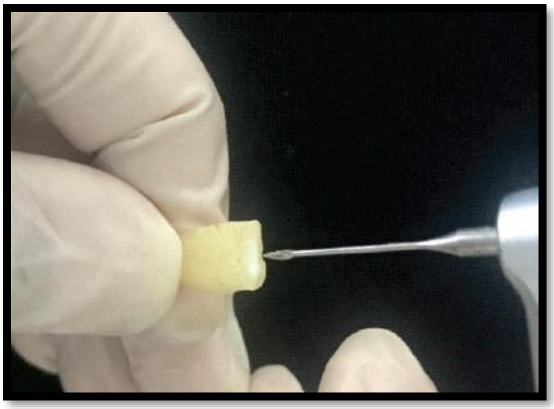


Decoronated Samples (n=20)

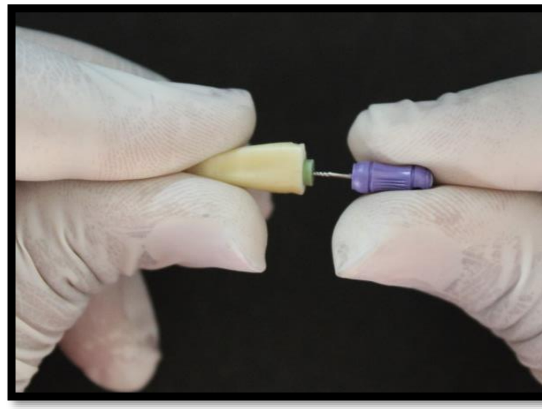
Length Measured with Digital Vernier Caliper

PLATE -XI

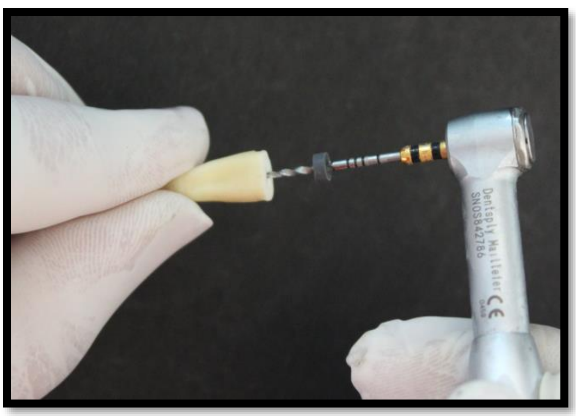
METHODOLOGY



Coronal Enlargement with Gates Glidden Drill



Working Length Determination



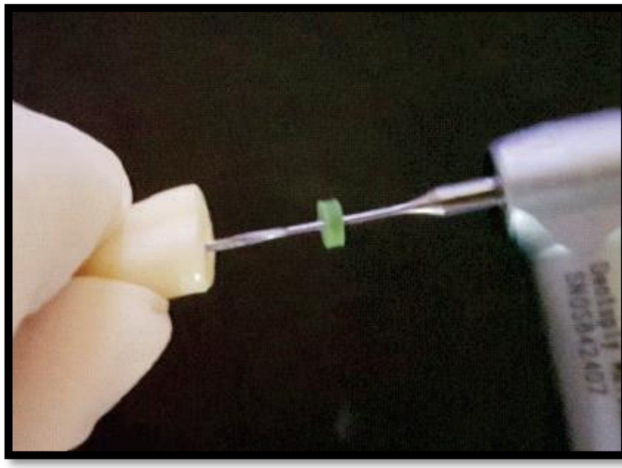
Biomechanical preparation

Irrigation of Root Canal

Master Cone Selection

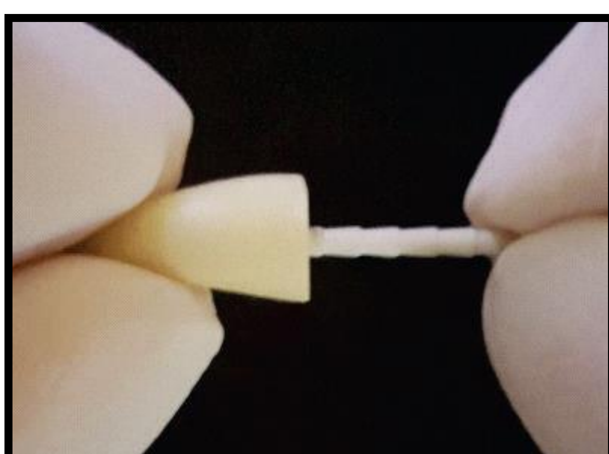
PLATE -XII

METHODOLOGY



Post Space Preparation

Post Space Irrigation

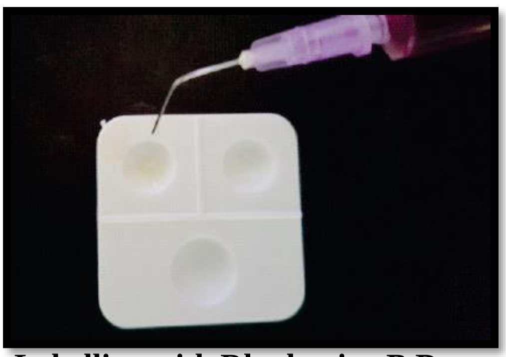


Trial of Fibre Post

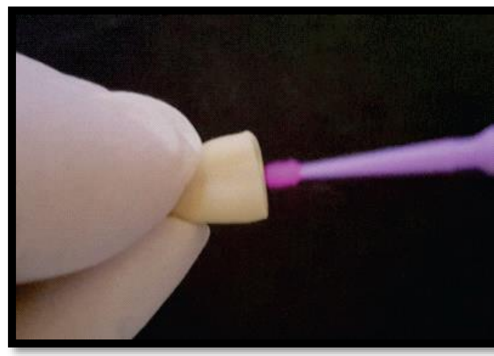
PLATE -XIII

METHODOLOGY

Dispensing and Mixing of Primer I and II



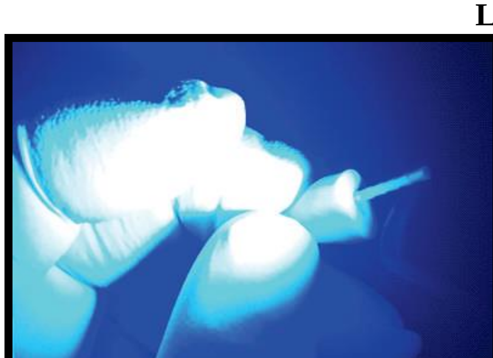
Labelling with Rhodamine B Dye



Application of Primer with Microbrush

Manipulation of Luting Cement

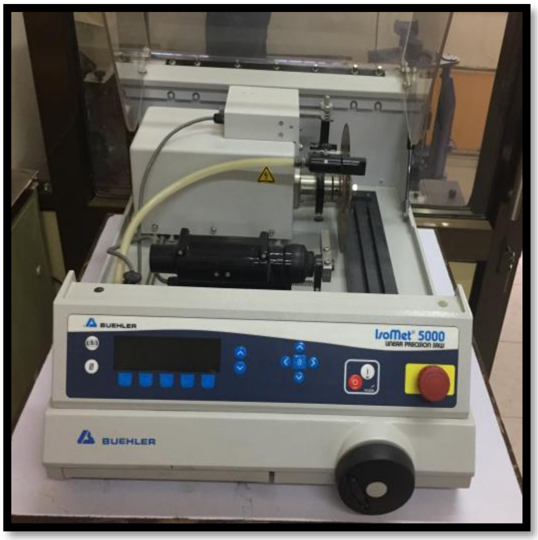
Application of Luting Cement with Lentulo Drill



Seating of Fibre Post and Curing

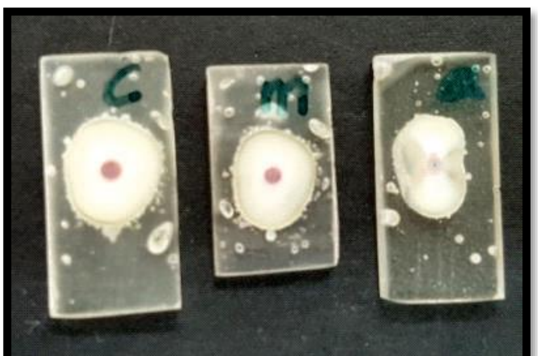
PLATE -XIV

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)

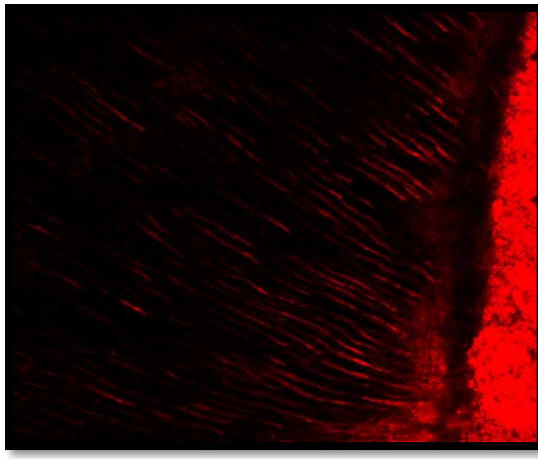
Universal Testing Machine (ACME Engineers, Model no. UNITEST-10)

PLATE -XV

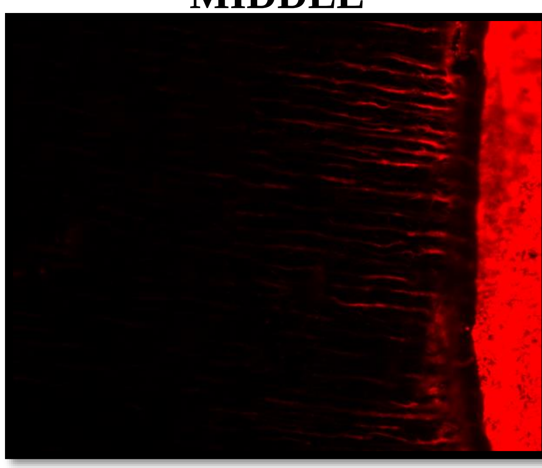
CONFOCAL LASER MICROSCOPIC IMAGES

GROUP - I

CORONAL



MIDDLE



APICAL

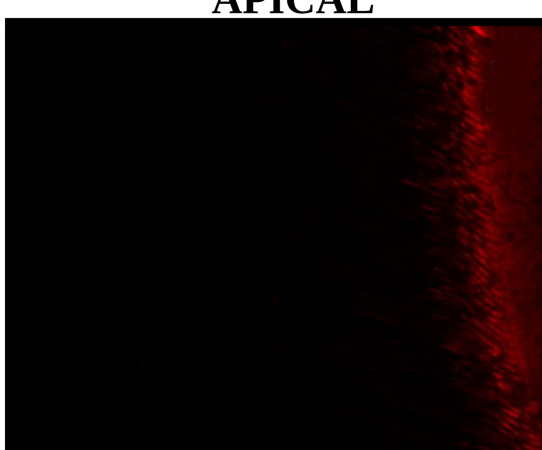
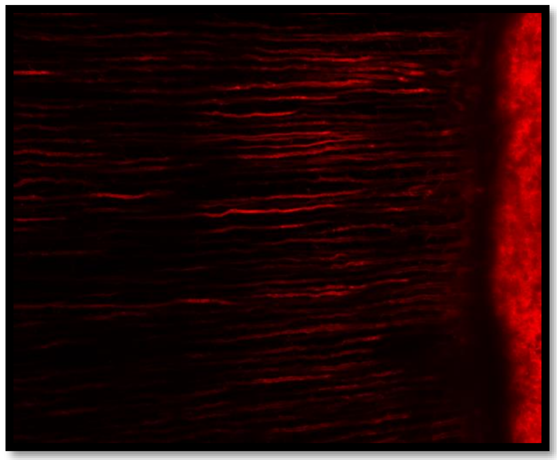


PLATE -XVI

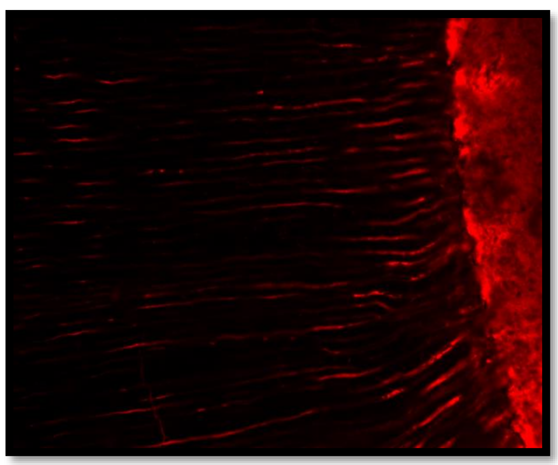
CONFOCAL LASER MICROSCOPIC IMAGES

GROUP - II

CORONAL



MIDDLE



APICAL

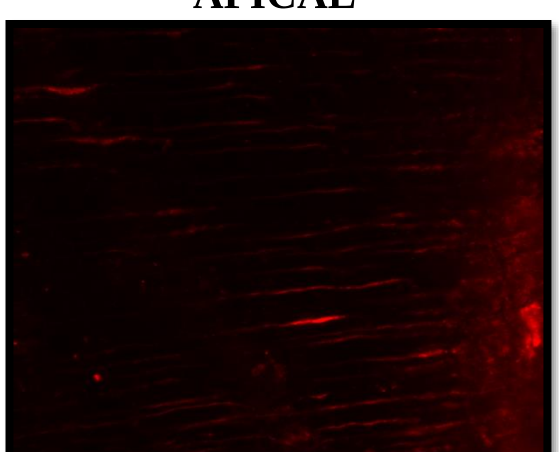
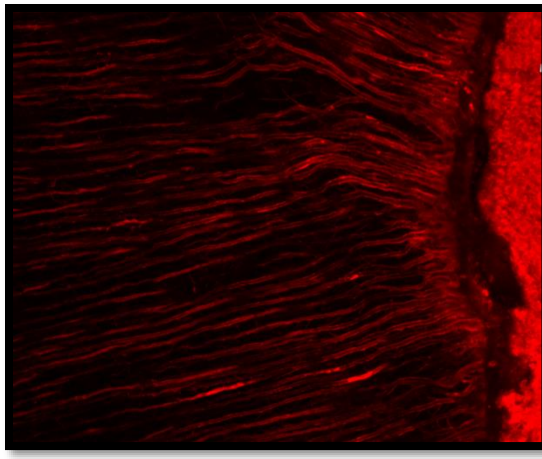


PLATE -XVII

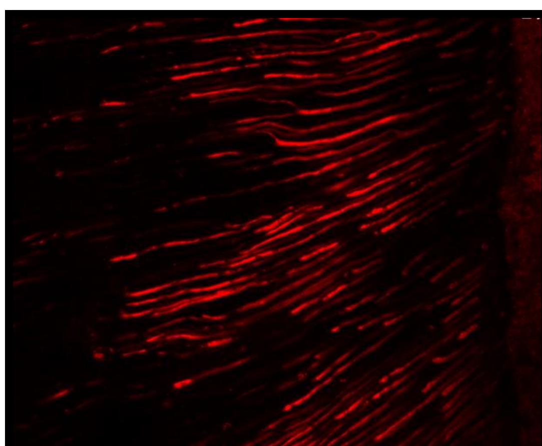
CONFOCAL LASER MICROSCOPIC IMAGES

GROUP - III

CORONAL



MIDDLE



APICAL

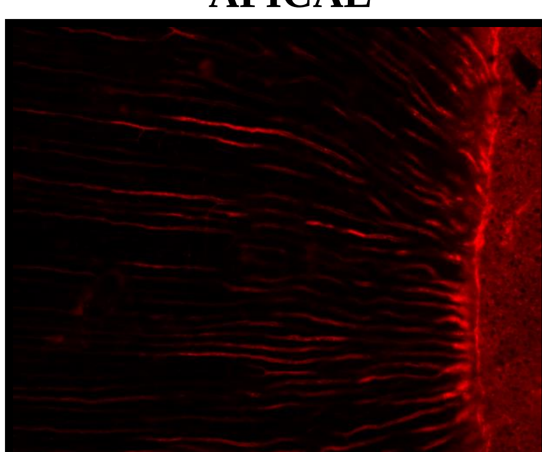
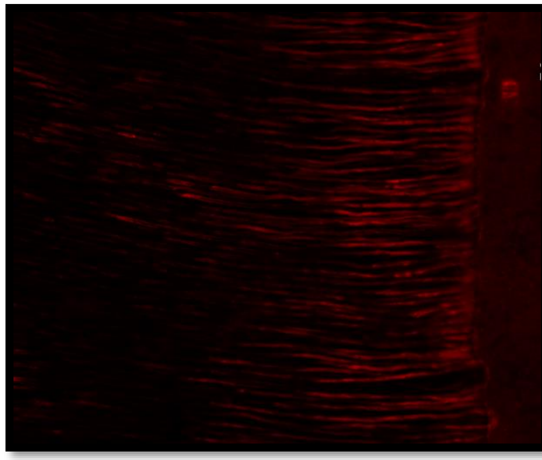


PLATE -XVIII

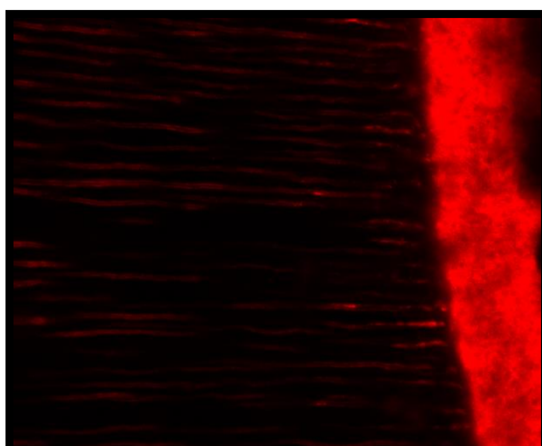
CONFOCAL LASER MICROSCOPIC IMAGES

GROUP - IV

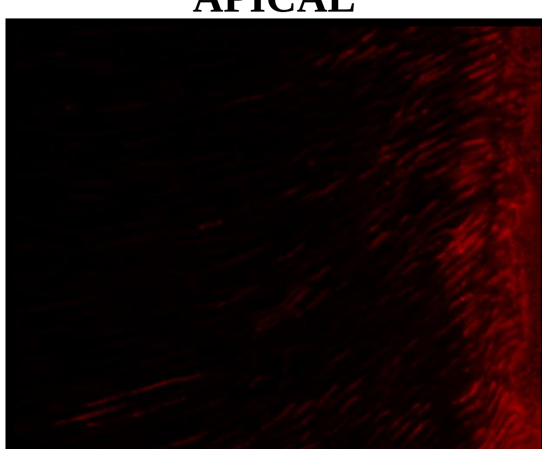
CORONAL



MIDDLE



APICAL



Results

The present in vitro study was carried out to evaluate the thickness of hybrid layer and number of resin tags at resin-dentin interface in the adhesive resin luting system after use of three different dentin modifiers by confocal laser scanning microscope and its relation to the push-out bond strength of fiber post to root dentin.

Depending upon the type of post space irrigation, the samples were randomly divided into four groups:

Group I: Canal irrigated for 1 min using 5 ml of 0.9% normal saline solution

Group II: Canal irrigated for 1 min using 5 ml of 0.9% normal saline solution followed by 1 min irrigation with 5 ml 17% EDTA.

Group III: Canal irrigated for 1 min using 5 ml of 0.9% normal saline solution followed by 1 min irrigation with 5 ml 7% Maleic acid.

G**roup IV:** Canal irrigated for 1 min using 5 ml of 0.9% normal saline solution followed by 1 min irrigation with 5 ml 0.2% Chitosan

Upon completion of post space irrigation, fibre posts were tried and cemented with the help of adhesive luting system.

All samples after post cementation, were sectioned at 1,4 and 7 mm of post space which represented the coronal, middle and apical sections of tooth respectively. These sections were evaluated for thickness of hybrid layer (μm), number of resin tags and push-out bond strength.

Statistical Analysis:

Licensed version of SPSS 20.0 (IBM Crop) was used for statistical analysis. The data on hybrid layer measurements at each section of tooth samples from four different treatment groups was summarized in terms of mean and standard deviation. The comparison of means was performed using a one-way analysis of variance (ANOVA). The pair-wise comparison of means between groups was carried out using Tukey’s post-hoc test. Intra-class correlation was used to decide the agreement between evaluators as regards the number of resin tags, while the kappa coefficient was obtained to arrive at the agreement between the multiple evaluators for scores. The number of resin tags and push-out bond strength were summarized and analyzed across groups using one-way ANOVA. The scores were summarized in terms of frequencies and the median scores for each group were used to compare the score differences across groups using the Kruskal-Wallis test. The paired comparison of scores between groups was carried out using the Wilcoxon rank-sum test.

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

The formulations used in the study are as below:

If x_1, x_2,…x_n are the observations on random variable X, then

A) **Sample mean** for a set of observations is given by

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

B) **Standard deviation** for a set of observations in 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

C) **Median**: It is the middle value of a set of values when arranged in the increasing order of magnitude.

D) **One-way Analysis of variance:** Analysis of variance (ANOVA) is used to test the significance of difference in the 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.

$$H_0 : \mu_1 = \mu_2 = ...\mu_m$$

against the alternative H_1 that they are not same.

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 is 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.

E) Kruskal-Wallis test

The test is a non-parametric equivalent of a one-way analysis of variance for comparing three or more groups. It is used for testing if the samples originate from the same or different populations. The procedure for determining the significance of difference across groups using the test is as below:

i) The n_1, n_2, …,n_k observations from k samples are combined into a single series of size n and arranged in order of magnitude from smallest to largest. The observations are then replaced by ranks from 1 assigned to smallest observation to n assigned to largest observation. When two or more observations have the same value, each observation is given a mean of the ranks for which it is tied.

ii) The ranks assigned to observations in each of the k groups are added separately to give *k* rank sums.

iii) The test statistic is defined as:

$$H = \frac{12}{n(n+1)} \sum_{j=1}^{k} \frac{R_j^2}{n_j} - 3(n+1)$$

where k is the number of groups; n_j is the number of observations in j^{th} group; n is the total number of samples from all the groups and R_j is the sum of ranks from j^{th} group.

iv) When there are more than 5 observations in one or more groups, H is compared with the tabulated value of χ^2 with k-1 degrees of freedom.

F) Wilcoxon rank-sum test

The test is a non-parametric equivalent of Student's t-test for independent samples when the assumption of normality is violated. It evaluates the null hypothesis that the two populations are the same against alternative that particular population has larger values than the other. It involves the computation of a test statistics based on ranked series. The observations are ranked according to magnitude irrespective of the two groups. The steps involved are as under:

i) Add the ranks for observations from group 1.

ii) Since the sum of all ranks equal $N (N+1)/2$, the sum of ranks in group 2 is the total sum minus the sum of group 1.

iii) A statistic U is defined as:

$$U_1 = R_1 - \frac{n_1(n_1+1)}{2}$$

where n_1 is the size of sample 1 and R_1 is the sum of the ranks of sample 1. Equally valid formula for U is

$$U_2 = R_2 - \frac{n_2(n_2+1)}{2}$$

The smaller of U_1 and U_2 is for significance testing.

For large sample sizes (N> 30), U is approximately normally distributed, and the standardized value is given by

$$z = \frac{U - m_U}{\sigma_U}$$

where,

m_U and σ_U are the mean and standard deviation of U. The significance of z can be obtained from normal probability tables. Here m_U and σ_U are given by:

$$m_U = \frac{n_1 n_2}{2}\,;\ \sigma_U = \sqrt{\frac{n_1 n_2(n_1+n_2+1)}{12}}$$

G) Intra-class correlation

Let $X_{1,j}$ and $X_{2,j}$ are the paired ratings provided by two experts on j=1,2,…,n subjects. Then for a two-way mixed-effect model with a single measurement type and absolute agreement, the formulation for intra-class correlation was:

$$ICC = \frac{MSR - MSE}{MSR + (k-1)MSE + k/n(MSC - MSE)}$$

Where, MSR is mean square for rows, MSE is mean square error, MSC is mean square for columns, n is the number of subjects and k is the number of raters/measurements.

H) Cohen's Kappa

Let the distribution of subjects according to ratings by two observers is as shown in the Table below:

Rater B	Rater A		Total
	1	2	
1	A	B	B_1=A+B
2	C	D	B_2=C+D
Total	A_1=A+C	A_2=B+D	N

Then, Cohen's Kappa as a measure of agreement between two raters is given by the expression: $Kappa = \frac{p_o - p_e}{1 - p_e}$

Where, $po = \frac{A+D}{N}$ and $p_e = \left(\frac{A_1}{N}\right)\left(\frac{B_1}{N}\right) + \left(\frac{A_2}{N}\right)\left(\frac{B_2}{N}\right)$

p_o measures the observed agreement between the two raters, while p_e estimates the chance that both the raters independently classify the subject into the same category.

Overall Results:

The mean values and standard deviations of thickness of hybrid layer, number of resin tags and push-out bond strength for four different irrigation protocol namely 0.9%Saline (Group I), 17% EDTA (group II), 7% Maleic acid (Group III) and 0.2% Chitosan (Group IV) at Coronal, Middle and Apical sections have been described in Table.1,Table.4 and Table.9 respectively.

The maximum thickness of hybrid layer (5.98± 0.49) was observed in Group III at coronal level whereas the minimum thickness of hybrid layer (1.92± 0.47) was observed in Group I at apical level. The maximum resin tags (25.12± 4.71) were observed in Group III at coronal level whereas the minimum resin tags (7.92± 2.43) were observed in Group I at apical level. The maximum push-out bond strength (12.23±1.64) was observed in Group III at coronal level whereas the minimum push-out bond strength (2.06±0.66) was observed in Group I at apical level.

Higher values for thickness of hybrid layer, no. of resin tags and push-out bond strength were obtained for group in which Maleic Acid was used as compared to those in which Saline, EDTA and Chitosan was used as post space irrigant. Higher values for thickness of hybrid, no. of resin tags and push-out bond strength were also obtained at coronal section as compared to middle and apical sections irrespective of irrigant used for post space irrigation (Table.1,Table.4 and Table.9, Figure 1, Figure 2 and Figure 4).

Analysis for Thickness of Hybrid Layer:

In order to determine the effect of post space irrigation (PSI) on thickness of hybrid layer, two-way analysis of variance was performed with groups as fixed effects. This analysis was performed at coronal, middle and apical sections, independently. (Table 1)

At coronal, the difference in the mean hybrid layer of four groups was significantly different as indicated by p-value < 0.0001, using one-way ANOVA. The paired comparison revealed that the mean for Group I was significantly lower than the other three groups with a p-value < 0.0001 using Tukey's post-hoc test. Also, the difference between groups II and III as well as group III and IV was significant with a p-value < 0.0001. Whereas, statistically insignificant results were seen between Group II and Group IV (p-value=0.146). (Table 2).

In the middle section, the difference of means across groups was statistically significant with a p-value < 0.0001 using one-way ANOVA. The mean for group I was the smallest and differed significantly from all other groups with a p-value < 0.0001. Also, the difference between group II and III as well as groups III and IV were significant with a p-value < 0.0001. Whereas, statistically insignificant results were seen between Group II and Group IV (p-value =0.995). (Table 2).

In the apical section, the difference of means across groups was statistically significant with a p-value < 0.0001 using one-way ANOVA. Again, the mean for group I was the smallest and differed significantly from all other groups with a p-value < 0.0001. Also, the difference between group II and III as well as group III and IV were significant with a p-value < 0.0001. Whereas, statistically insignificant results were seen between Group II and Group IV (p-value =0.800). (Table 2).

Analysis for Number of Resin Tags:

In order to determine the effect of post space irrigation (PSI) on no. of resin tags, two-way analysis of variance was performed with Groups as fixed effects. This analysis was performed at coronal, middle and apical sections, independently. (Table 4)

At coronal, the mean number of resin tags across groups differed significantly as indicated by p-value < 0.0001, using one-way ANOVA. The paired comparison revealed that the mean for Group I was significantly lower than the other three groups with a p-value < 0.0001 using Tukey's post-hoc test. The paired differences between Groups II, III and IV were statistically insignificant (Table 5).

In the middle section, the difference of means across groups was statistically significant with a p-value < 0.0001 using one-way ANOVA. The mean for Group I was the smallest and differed significantly from all other groups with a p-value < 0.0001. The differences between groups II, III and IV were statistically insignificant (Table 5).

In the apical section, the difference of means across groups was statistically significant with a p-value < 0.0001 using one-way ANOVA. Again, the mean for Group I was the smallest and differed significantly from Groups II, III and IV with p-values 0.037, < 0.0001 and 0.027 respectively. Also, the difference between group II and III as well as groups III and IV were significant with a p-value < 0.0001.

Whereas, statistically insignificant results were seen between Group II and Group IV (p-value =0.999) (Table 5).

Analysis for Resin Tags Score:

The kappa coefficient was used on scores provided by two observers, which ranged from 0.64 to 0.79 indicating substantial agreement between the two observers.

Table 7 shows the frequencies and the median score for tooth samples according to sections in four study groups.

In the coronal section, the median scores across groups differed significantly as indicated by p-value < 0.0001, using the Kruskal-Wallis test. The paired comparison revealed that the distribution of scores in Group I was significantly lower than the other three groups with a p-value < 0.0001 using the Wilcoxon rank-sum test. The paired differences between other groups were statistically insignificant (Table 8).

In the middle section, the differences in scores across groups were statistically significant with a p-value < 0.0001. The median for Group I was the smallest and differed significantly from Groups II, III and IV with p-value < 0.0001, < 0.0001 and 0.003 respectively (Table 8).

In the apical section, the differences in scores across groups were statistically significant with a p-value < 0.0001. Again, the median for Group I was the smallest and differed significantly from groups II, III and IV with p-values < 0.0001, 0.001

and 0.002 respectively. Also, the difference between groups II and IV was significant with a p-value of 0.025 (Table 8).

Analysis for Push-Out Bond Strength:

In order to determine the effect of post space irrigation (PSI) 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. (Table 9)

At coronal, the mean push-out bond strength across groups differed significantly as indicated by p-value < 0.0001, using one-way ANOVA. The paired comparison revealed that the mean for Group I was significantly lower than the other three groups with a p-value < 0.0001 using Tukey’s post-hoc test. The paired differences between Groups II and III, as well as Groups III and IV were statistically significant with a p-value < 0.0001. Whereas, statistically insignificant results were seen between Group II and Group IV (p-value =0.877). (Table 10)

In the middle section, the difference of means across groups was statistically significant with a p-value < 0.0001 using one-way ANOVA. The mean for Group I was the smallest and differed significantly from groups II, III and IV with p-value < 0.0001, < 0.0001 and 0.001 respectively. Also, Groups II and III, as well as groups III and IV differed significantly with p-values 0.009 and < 0.0001 respectively. Whereas, statistically insignificant results were seen between Group II and Group IV (p-value =0.548). (Table 10)

In the apical section, the difference of means across groups was statistically significant with a p-value < 0.0001 using one-way ANOVA. Again, the mean for Group I was the smallest and differed significantly from other groups with a p-value < 0.0001. Also, the difference between Group II and III as well as groups III and IV were significant, each with a p-value of 0.001. Whereas, statistically insignificant results were seen between Group II and Group IV (p-value =0.999). (Table 10)

Discussion

Post endodontic restoration is of vital importance for a tooth to act as functional unit within the oral cavity after successful root canal therapy. Many researchers in their studies have stated that the post endodontic restoration has a greater impact on the outcome of endodontic therapy than the endodontic filling itself.[53-56]

Teeth with a significant loss of coronal tooth structure often require a post and core system.[57,58] Traditionally, cast post-and-core was the most commonly used post type. However, there are several disadvantages associated with conventional cast post-and-cores such as: loss of post retention, root fractures, and risk of corrosion.

Later, introduction of fiber post brought a revolution in the field of dentistry by providing a reliable substitute for metallic post. The first evidence on use of dental fiber posts was given by **Duret et al. (1990).**[31] Fiber posts are cemented into the root dentine with the help of adhesive resins to avoid frictional retention between the post and root dentin.[59]

Although number of factors like interpenetration, micro-mechanical interlocking and chemical bonding contribute in effective bonding between post and dentin. **Ferrari M & Davidson C (1996)** stated that the overall bond strength of resin cement to dentin is regarded by two fundamental processes involved: First, formation of hybrid layer, and second intra-tubular penetration of adhesive i.e. resins tags.[60] According to **Nakabayashi N et al. (1992)** the interdiffusion zone of demineralized intertubular and peritubular dentine and polymerized resin is the key element in developing an ideal bond between adhesive and substrate.[61]

Mineralized dentin usually does not allow much diffusion of monomer into its tubules. Furthermore, prepared dentinal walls are covered with smear layer which further hinders the penetration of monomer into dentin. Thus, dentin must be suitably conditioned or modified to permit diffusion of monomer into demineralized collagen matrix.[62]

EDTA is the most widely used in post-space irrigation and complements the cleaning of canal space by acting on inorganic material. However, **Khedmat S and Shokouhinejad N (2008)** reported that the application of EDTA for more than a minute and volume more than 1 ml results in dentinal erosion.[63] Therefore, a continuous search for more biocompatible solution to EDTA, has lead to application of several weak acids and other naturally occurring compounds in endodontic irrigation.

Previous researchers have shown that bonding to root canals may be influenced by the post space irrigants used prior to post cementation. 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 post space irrigation on hybrid layer and resin tag formation and its relation with push-out bond strength. Hence, the following study was carried out to evaluate the hybrid layer and resin tags at resin dentin interface after use of three different dentin modifiers (EDTA, Maleic Acid and Chitosan) using Confocal Laser Scanning Microscopy (CLSM) and its relation to push-out bond strength.

One hundred freshly extracted single rooted human madibular first premolars with single canal were selected for the study. The teeth were cleaned and disinfected as per the recommendations and guidelines laid down by OSHA and CDC.[50] The presence of single canal was confirmed by bucco-lingual and mesio-distal radiographs of the sample. The teeth were collected and stored in phosphate-buffered saline for not more than 12 weeks as suggested by **Jameson MW et al (1994).[51]** They observed that the extracted tooth get affected due to water loss with dehydration of dentin. This is further affected by the type of storage media used and the duration for which it is stored. Phosphate-buffered saline shows the best compatibility in maintaining the hydration of the extracted teeth.

For sample size estimation, a study by **Fan F et al. (2017)[21]** was referred. Higher bond-strength was seen in Maleic acid group (12.5± 1.5) at coronal level and least was seen in saline group (5.84 ± 0.71) 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 25 samples per group. Therefore, the total sample size for the current research was kept 100.

The formulation used was:

$$n = \frac{2z^2_{\alpha/2}\,\sigma^2}{\varepsilon^2}$$

Where $z_{\alpha/2}$ was critical value for 95% significance level; σ^2 is the pooled standard deviation and ε is the permissible error or effect size.

Mandibular First Premolars 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.[2] All these factors predispose the tooth to traumatic dental injuries. Crown fractures are the most common of all dental injuries and often require placement of fiber posts. 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.

Method described by **Shanmugaraj M et al. (2007)** on extracted teeth for measurement of working length was employed in this study. He advocated the insertion of 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).[64]

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)**[65] 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.**[66] 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 2.5% NaOCl using a 30 gauge needle..

Root canal sealers are used in combination with gutta percha for an endodontic obturation. It is difficult to completely remove the sealer from the surface of root canal dentin, while post space preparation. Sealers can thus directly or indirectly influence resin-dentin bonding. **Demiryürek EO et al. (2010)**[67] showed that the root canal sealers, based on their chemical composition, have an effect on bonding of fibre posts to root canal dentin. Thus, root canal was obturated by means of sectional obturation technique up to 5mm from the apex using a resin based sealer AH plus. After obturation, the post space preparation was done with peeso drills and a post space depth of 10 mm was kept for all the samples for standardization. In this study, post space was prepared till size #3 peeso drills as their rotary action creates parallel walls, which provide optimal retention for the post.

Czonstkowsky et al. (1990)[25] observed that the motor-driven instruments, such as peeso post drills, produced quantitatively more smear layer than hand files. **Serafino C et al. (2004)**[30] stated that this thick smear layer could affect the bonding of fibre post to root canal dentin hence should be removed. Researcher have shown that just rinsing the post space with water or saline alone before bonding might not be sufficient for removal of smear layer.[68] Thus it is necessary to achieve a clean, smear layer free post space area for optimal rehabilitation of post in the canal space.

In the present study, after post space preparation, block randomization of samples was done into four groups corresponding to treatment with different irrigant solutions:

Group I: Canal irrigated for 1 min using 5 ml of 0.9% normal saline solution

Group II: Canal irrigated for 1 min using 5 ml of 0.9% normal saline solution followed by 1 min irrigation with 5 ml 17% EDTA.

Group III: Canal irrigated for 1 min using 5 ml of 0.9% normal saline solution followed by 1 min irrigation with 5 ml 7% Maleic acid.

Group IV: Canal irrigated for 1 min using 5 ml of 0.9% normal saline solution followed by 1 min irrigation with 5 ml 0.2% Chitosan

The residual chemical irrigants and their products are likely to diffuse into the dentinal tubules, which may affect the infiltration of the resin into the demineralised dentin or interfere with the complete polymerisation of the adhesives. Hence, in our research upon completion of the respective irrigation protocol, the canals were thoroughly rinsed with distilled water and dried with paper points. [13,69,70]

In present study, 0.1% Rhodamine B dye was mixed with equal drops of Prime and Bond adhesive and self cure activator The luting of fibre posts with the Prime and Bond NT and Calibra cement was done according to manufacturer's manual. **Monticelli F et al. (2006)[71]** 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.

In the current study, after luting of fibre posts, the samples were sectioned at 1,4 and 7 mm from CEJ. Each section represented the coronal, middle and apical part of the post space preparation. As studies on the morphology of root canal dentin showed that number of tubules decreases from the crown to the apex, the response to post space irrigation and consequently dentine bonding can vary among different areas of the same root canal.[72,73] The samples were kept in a humidifier.

To reduce the variability, all the samples were prepared and investigated by one operator using the standard technique. However, the evaluation and scoring of resin tags were performed by two evaluators who were blinded about the irrigation protocol used to avoid observer bias.

In the present study, thickness of hybrid layer and number of resin tags were compared after the use of different dentin modifiers, at different regions of tooth under CLSM. Also, its relation to push-out bond strength was evaluated.

1) Effect of post-space irrigation on resin dentin interphase and number of resin tag formation:

Most promising approach to provide bonding to dentin is through formation of intimate mechanical interlocking between partially demineratized dentin and dentin bonding agents called as resin-dentin interdiffusion zone (also known as hybrid layer formation). Both SEM and TEM confirmed the presence of the resin-dentin interdiffusion zone.[74]

Though, several microscopy techniques are currently used to evaluate the resin dentin interface and resin tags. Introduction of Confocal laser scanning microscopy (CLSM), used in combination with fluorescent dyes, has provided a valuable visualization of bonding structures at relative low magnification as 100X 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 indispensible 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.[75] Thus, in the current research CLSM was preferred over SEM and TEM and the sections were observed under the confocal laser scanning microscope.

In the present study, the samples were evaluated and scores were allocated to each sample. The Kappa co-efficient was used on score provided by two observers which ranged between 0.64 to 0.79. These findings were in accordance with the study done by **Fagundes T et al. (2014)**[52] The inter-examiner correlation for number of resin tags was more than 0.95 indicating very good agreement between the observers.

In this study, maximum thickness of hybrid layer **(5.98±0.49)** was observed in Maleic acid group as compared to other groups and the mean thickness of hybrid layer was lowest for Group I and differed significantly from all other groups with a p value < **0.0001** at coronal, middle and apical levels. Whereas, a non significant difference in thickness of hybrid layer was observed in Group II and Group IV. **(p value = 0.146, 0.995, 0.800)** at coronal, middle and apical levels respectively.

Highly significant difference for no. of resin tags were obtained for groups in which Maleic acid was used as compared to those where post space was irrigated with EDTA and Chitosan at apical level only **(p-value < 0.0001).** However, a non significant difference in values of no. of resin tags was observed between the three groups (Maleic acid, EDTA and Chitosan) at coronal and middle levels.

The results are in accordance to the study done by **Ulusoy O and Gorgul G (2013)**[76] where they stated that EDTA removes the smear layer efficiently only in the coronal and middle thirds of the root canal, whereas MA successfully removes the smear layer also from the apical third.

Better results for Maleic acid could be justified as Maleic acid (MA) is a soft organic acid that is used as an acid conditioner in adhesive dentistry, because of its strong physicochemical action on both organic and inorganic particles.[77] **Haapasalo M et al. (2010)** stated that EDTA is effective at dissolving inorganic particles and has little or no effect on organic tissue.[17] Similarly, Chitosan acts on the inorganic portion of smear layer only.[22]

The results of this study also showed a decrease in the number of resin tags from the coronal to the apical region of the prepared root canal space. This is in accordance with the findings of **Ferrari M et al. (2000)**[73] who observed a significantly higher density of dentinal tubules in the coronal third of the root canal than in the middle and apical thirds. **Mjör A et al. (2000)**[78] also reported a decreased number of dentinal tubules from about 40,000 per mm^2 in the coronal region of the root canal to 14,400 per mm^2 in the apical region. They thus stated that the formation of hybrid layer would be of more importance for adhesion to apical dentin than resin tag formation, because fewer tags are available for resin penetration in this area.

Another explanation for the decrease in no. of resin tags could be morphological variations that impact the resin penetration in an apical direction. The diameter of the dentinal tubules are also larger cervically than apically as well as sclerotic processes, can hamper the access to the dentinal tubules.[79,80]

It also has to be considered that bonding to root canal dentin might be hampered by a lack of direct vision and luting agent application techniques.[81] In this study, poorer tag formation in the apical third might be due to the fact that conditioning with a microbrush would be better in the cervical region whereas in the apical third the contact and fluid exchange might be reduced, resulting in resin penetration less deeply into the tubules. These findings are in accordance with the results of **Ferrari M et al. (2002).**[82]

2) Effect of post-space irrigation on push-out bond strength:

For determining the bond strength of fiber–post to root dentin, push-out bond strength has been reported to be more effective than the microtensile strength.[83]

Thus, the former 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.[41]

Higher values for bond strength were obtained for groups where Maleic acid was used as a post space irrigant as compared to those with Saline, EDTA and Chitosan, irrespective of the sections.

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 III (**12.23 ± 1.64, 7.35 ± 1.82 and 4.58 ± 1.27)** in coronal, middle and apical section respectively and lowest mean push-out bond strength value was seen in Group I **(4.83 ± 1.53, 3.90 ± 1.72 and 2.06 ± 0.66)** in coronal, middle and apical section respectively. Whereas, a non significant difference in push-out bond strength was observed in Group II and Group IV.

Serafino C et al. (2004) stated that the action of the drills used to create post space, produces a new smear layer rich in sealer and gutta-percha remnants plasticized by the friction heat of the drill. This diminishes the penetration and chemical action of the monomers in resin adhesives.[30]

Previous studies have reported that use of various chemical agents can be used to increase the micromechanical retention of cement by removing the smear layer.[19,20,21] Thus, the difference in the push-out bond strength should be closely related to the ability of different irrigants to remove the smear layer.

In the present study the lowest bond strength values were obtained in Control group because post surface was not altered by any surface treatment. Thus the result obtained in the study confirms that Chemical agents used for irrigation have a positive effect on smear layer removal and thus increases the push-out bond strength of the fiber post to root dentine.

These results are in accordance with the study done by **Fan F et al. (2017)** where they concluded that the bond strength of fiber posts to root dentin varies according to the irrigation protocol and that the irrigation of post space with Maleic acid significantly improves the bond strength of fiber post to root dentin.[21]

The results are also in accordance with what is shown by **Ballal et al. (2009)** according to which the final irrigation with MA is more effective than EDTA in removal of smear layer.[19]

Also better results were obtained at coronal and middle thirds as compared to apical thirds which may be due to the fact that a larger canal diameter in the coronal and middle third exposes the dentin to a higher volume of irrigants. Thus, improving the efficacy of smear layer removal which ultimately leads to higher bond strength.

3) Correlation between number of resin tags formed and push-out bond strength:

A higher value for thickness of hybrid layer, no. of resin tags and push out bond strength is observed in the group where maleic acid was used as a post-space irrigant. Thus, showing a close relationship between push-out bond strength and no. of resin tags. This results are in contrast to the study done by **Crivano E et al.(2014)**[84] where they stated that resin tags have no contribution on push-out bond strength of self-adhesive resin cement. However, the surface treatment in the above study was different from the present study. Hence, more research needs to be carried out in this area of correlation between number of resin tags formed and push-out bond strength.

Thus the hypothesis of this study that there would be no significant difference on the push out bond strength of luted fibre post to root dentin when treated with 17% ethylenediaminetetraactetic acid (EDTA), 7% Maleic acid and 0.2% Chitosan and has no relation to the number of resin tag formation was rejected. The hybrid layer thickness, number of resin tags and push-out bond strength of the bonding agent differed significantly amongst the four groups.

Therefore, within the limitations of the study it can be concluded that significantly better hybrid layer, resin tags and greater push-out bond strength is obtained when the post space is irrigated with Maleic Acid.

Limitations

Inspite of stringent care taken in every step of the 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 prefabricated posts offer better prognosis of endodontically treated teeth than the conventional cast posts. Fibre posts are chemically compatible with Bis-GMA based luting agents and thus are cemented using an adhesive technique. However, several researchers have evaluated the efficacy of fibre posts and identified debonding along the resin dentin interface as the most frequent mode of failure.

Resin tags and Hybrid layer form the base of micromechanical unit of dental adhesive technique. The homogeneity of this micromechanical unit is influenced by endodontic therapy, post space preparation and irrigation, the adhesive luting systems used and the location in root canal.

The present in vitro study was carried out to evaluate the hybrid layer and resin tags at resin dentin interface after use of three different dentin modifiers using a confocal laser scanning microscope and its relation to push-out bond strength of fiber post to root dentin.

In present study, 100 freshly extracted human mandibular first premolars, which fulfilled the inclusion criteria, were selected. The teeth were decoronated, prepared and obturated using standard techniques. Post space preparation was done and depending on the post space irrigation protocol, the teeth were divided into four groups of 25 each:

Group I: Canal irrigated for 1 min using 5 ml of 0.9% normal saline solution

Group II: Canal irrigated for 1 min using 5 ml of 0.9% normal saline solution followed by 1 min irrigation with 5 ml 17% EDTA.

Group III: Canal irrigated for 1 min using 5 ml of 0.9% normal saline solution followed by 1 min irrigation with 5 ml 7% Maleic acid.

G**roup IV:** Canal irrigated for 1 min using 5 ml of 0.9% normal saline solution followed by 1 min irrigation with 5 ml 0.2% Chitosan**.**

After thorough irrigation with distilled water and complete drying of canal space, fibre posts were tried and luted using the adhesive luting system. All samples were then sectioned using a precision saw at 1,4 and 7 mm of the post space. These sections were evaluated for the thickness of hybrid layer and no. of resin tags under confocal laser scanning microscope and the same samples were evaluated for push-out bond strength under universal testing machine.

The results obtained indicated that there is a highly significant difference in thickness of hybrid layer, no. of resin tags and push-out bond strength when the three different dentin modifier 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 thickness of hybrid layer, no. of resin tags and bond strength of fibre posts to root dentine varies according to the irrigation regimen

2. Irrigation with Maleic acid (MA) significantly improves the thickness of hybrid layer, no. of resin tags and bond strength of fibre posts to root dentine

3. The thickness of hybrid layer, no. of resin tags and push-out bond strength significantly decreased from cervical to apical level of post space.

Taking into consideration the findings of the present study, it can be concluded that under experimental conditions, a thicker hybrid layer, higher no. of resin tags and higher bond strength of fibre posts to root dentine are present when the post space is irrigated with MA. However, further investigations which could give a conclusive remark on the long-term effect of MA on the bond strength of fibre post to root dentine are needed.

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Tables & Graphs

Table 1: Descriptive statistics i.e Mean and Standard Deviation for thickness of hybrid layer according to groups (Saline, EDTA, Maleic Acid and Chitosan) at Coronal, Middle and Apical sections.

Section	Hybrid Layer (μm)								P-value*
	Group I (n=25)		Group II (n=25)		Group III (n=25)		Group IV (n=25)		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Coronal	2.25	0.49	4.92	0.68	5.98	0.49	5.27	0.59	< **0.0001 (S)**
Middle	2.22	0.72	4.07	0.55	5.70	0.47	4.02	0.77	< **0.0001 (S)**
Apical	1.92	0.47	3.39	0.66	5.43	0.46	3.56	0.92	< **0.0001 (S)**

*Obtained using ANOVA; S: Significant

Table 2: Paired comparison of hybrid layer between groups at three sections:

Section	Groups		Absolute Mean Difference	P-value
Coronal	I	II	2.67	< **0.0001 (S)**
		III	3.72	< **0.0001 (S)**
		IV	3.01	< **0.0001 (S)**
	II	III	1.05	< **0.0001 (S)**
		IV	0.35	0.146
	III	IV	0.71	< **0.0001 (S)**
Middle	I	II	1.84	< **0.0001 (S)**
		III	3.47	< **0.0001 (S)**
		IV	1.80	< **0.0001 (S)**
	II	III	1.63	< **0.0001 (S)**
		IV	0.04	0.995
	III	IV	1.67	< **0.0001 (S)**
Apical	I	II	1.48	< **0.0001 (S)**
		III	3.51	< **0.0001 (S)**
		IV	1.64	< **0.0001 (S)**
	II	III	2.03	< **0.0001 (S)**
		IV	0.17	0.800
	III	IV	1.87	< **0.0001 (S)**

*Obtained using Tukey's HSD test; Bold value indicates a significant difference

Table 3: Intraclass correlation between two observers for count of number of resin tags:

Groups	Section	Intra Class Correlation Coefficient	P-value*
Saline	Coronal	0.950	< **0.0001 (S)**
	Middle	0.956	< **0.0001 (S)**
	Apical	0.987	< **0.0001 (S)**
EDTA	Coronal	0.958	< **0.0001 (S)**
	Middle	0.953	< **0.0001 (S)**
	Apical	0.982	< **0.0001 (S)**
Maleic Acid	Coronal	0.934	< **0.0001 (S)**
	Middle	0.973	< **0.0001 (S)**
	Apical	0.975	< **0.0001 (S)**
Chitosan	Coronal	0.965	< **0.0001 (S)**
	Middle	0.937	< **0.0001 (S)**
	Apical	0.994	< **0.0001 (S)**

*S: Significant

Table 4: Descriptive statistics i.e Mean and Standard Deviation for number resin tags according to groups (Saline, EDTA, Maleic Acid and Chitosan) at Coronal, Middle and Apical sections.

Section	No. of resin tags								P-value*
	Group I		Group II		Group III		Group IV		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Coronal	15.56	4.21	23.88	4.56	25.12	4.71	23.48	3.96	< **0.0001 (S)**
Middle	11.08	3.25	20.56	3.18	21.80	3.40	20.16	3.50	< **0.0001 (S)**
Apical	7.92	2.43	11.44	5.21	17.72	4.74	11.60	5.24	< **0.0001 (S)**

*Obtained using ANOVA; S: Significant

Table 5: Paired comparison of number of resin tags between groups at three sections:

Section	Groups		Absolute Mean Difference	P-value
Coronal	I	II	8.32	**< 0.0001 (S)**
		III	9.56	**< 0.0001 (S)**
		IV	7.92	**< 0.0001 (S)**
	II	III	1.24	0.748
		IV	0.40	0.988
	III	IV	1.64	0.548
Middle	I	II	9.48	**< 0.0001 (S)**
		III	10.72	**< 0.0001 (S)**
		IV	9.08	**< 0.0001 (S)**
	II	III	1.24	0.556
		IV	0.40	0.974
	III	IV	1.64	0.309
Apical	I	II	3.52	**0.037 (S)**
		III	9.80	**< 0.0001 (S)**
		IV	3.68	**0.027 (S)**
	II	III	6.28	**< 0.0001 (S)**
		IV	0.16	0.999
	III	IV	6.12	**< 0.0001 (S)**

*Obtained using Tukey's HSD test; Bold value indicates a significant difference

Table 6: Kappa coefficient showing agreement between two observers for scores:

Groups	Section	Kappa coefficient	P-value*
Saline	Coronal	0.667	**0.001 (S)**
	Middle	0.706	**< 0.0001 (S)**
	Apical	0.756	**< 0.0001 (S)**
EDTA	Coronal	0.686	**< 0.0001 (S)**
	Middle	0.743	**< 0.0001 (S)**
	Apical	0.920	**< 0.0001 (S)**
Maleic Acid	Coronal	0.675	**0.001 (S)**
	Middle	0.640	**< 0.0001 (S)**
	Apical	0.804	**< 0.0001 (S)**
Chitosan	Coronal	0.693	**< 0.0001 (S)**
	Middle	0.876	**< 0.0001 (S)**
	Apical	0.802	**< 0.0001 (S)**

*S: Significant

Table 7: Median scores for each section and for four groups:

Section	Score								P-value*
	Group I (n=25)		Group II (n=25)		Group III (n=25)		Group IV (n=25)		
	Count	Median	Count	Median	Count	Median	Count	Median	
Coronal									
1	20		3		1		2		
2	5	1	15	2	13	2	14	2	**< 0.0001 (S)**
3	0		7		11		9		
Middle									
1	23		4		5		11		
2	2	1	19	2	16	2	11	2	**< 0.0001 (S)**
3			2		4		3		
Apical									
0	6		0				0		
1	19	1	15	1	15		20	1	**< 0.0001 (S)**
2	0		10		7	1	5		
3	0		0		3		0		

*Obtained using Kruskal-Wallis test; S: Significant

Table 8: Paired comparison of scores between groups at three sections:

Sections	Groups		P-value*
Coronal	I	II	**< 0.0001 (S)**
		III	**< 0.0001 (S)**
		IV	**< 0.0001 (S)**
	II	III	0.180
		IV	0.467
	III	IV	0.083
Middle	I	II	**< 0.0001 (S)**
		III	**< 0.0001 (S)**
		IV	**0.003 (S)**
	II	III	0.813
		IV	0.186
	III	IV	0.405
Apical	I	II	**< 0.0001 (S)**
		III	**0.001 (S)**
		IV	**0.002 (S)**
	II	III	0.519
		IV	**0.025 (S)**
	III	IV	0.096

*Obtained using Wilcoxon rank-sum test; Bold value indicates significant difference

Table 9: Descriptive statistics i.e Mean and Standard Deviation for push-out bond strength according to groups (Saline, EDTA, Maleic Acid, Chitosan) at Coronal, Middle and Apical sections

Sections	Push-out bond strength (MPa)								P-value*
	Group I		Group II		Group III		Group IV		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Coronal	4.83	1.53	9.87	1.17	12.23	1.64	10.18	1.57	**< 0.0001 (S)**
Middle	3.90	1.72	6.04	0.72	7.35	1.82	5.50	1.21	**< 0.0001 (S)**
Apical	2.06	0.66	3.61	0.63	4.58	1.27	3.64	0.67	**< 0.0001 (S)**

*Obtained using ANOVA; S: Significant

Table 10: Paired comparison of push-out bond strength between groups at three sections:

Section	Groups		Absolute Mean Difference	P-value
Coronal	I	II	5.04	< **0.0001 (S)**
		III	7.40	< **0.0001 (S)**
		IV	5.35	< **0.0001 (S)**
	II	III	2.37	< **0.0001 (S)**
		IV	0.32	0.877
	III	IV	2.05	< **0.0001 (S)**
Middle	I	II	2.14	< **0.0001 (S)**
		III	3.45	< **0.0001 (S)**
		IV	1.60	**0.001 (S)**
	II	III	1.31	**0.009 (S)**
		IV	0.54	0.548
	III	IV	1.85	< **0.0001 (S)**
Apical	I	II	1.55	< **0.0001 (S)**
		III	2.52	< **0.0001 (S)**
		IV	1.58	< **0.0001 (S)**
	II	III	0.96	**0.001 (S)**
		IV	0.03	0.999
	III	IV	0.94	**0.001 (S)**

*Obtained using Tukey's HSD test; Bold value indicates a significant difference

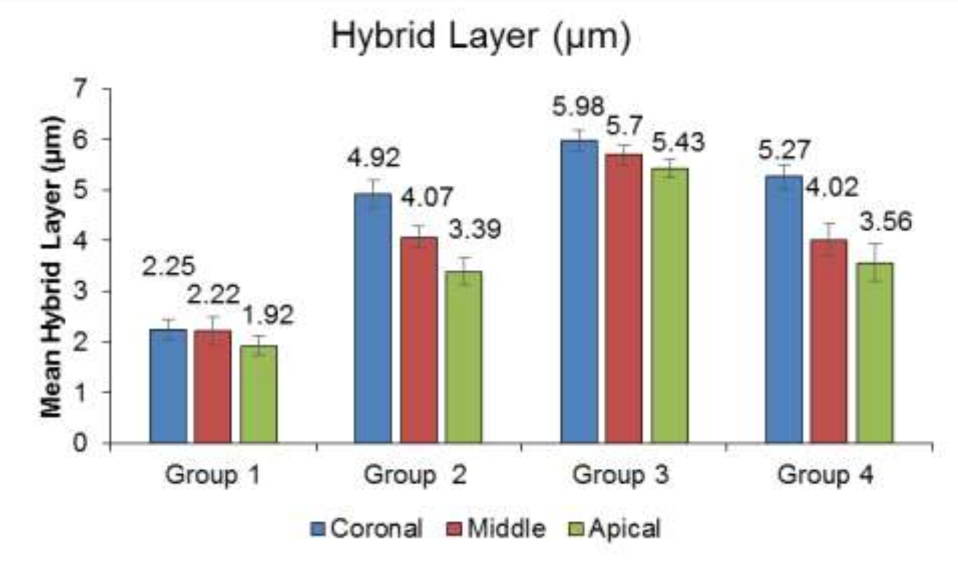

Figure 1: Column chart with error bars showing mean hybrid layer at each section according to group

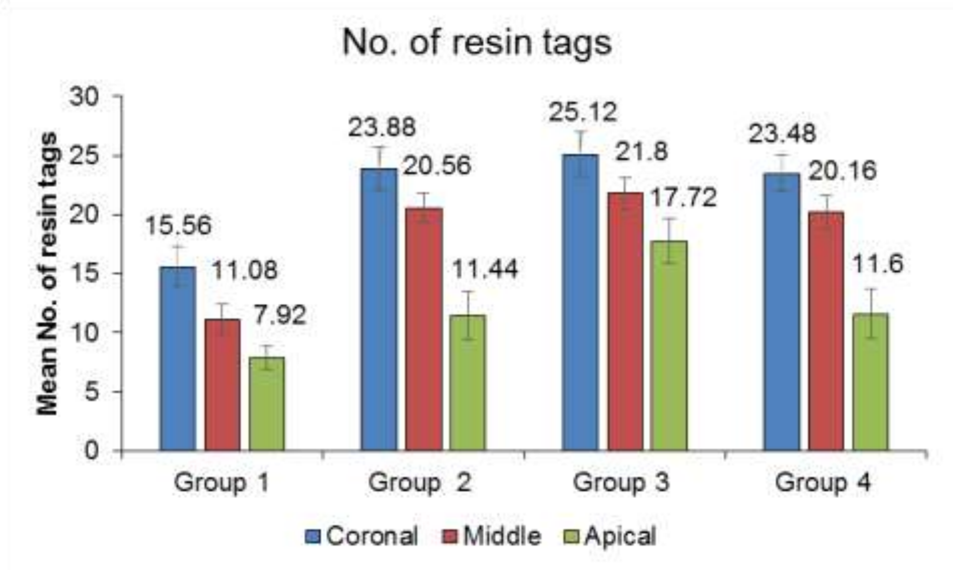

Figure 2: Column chart with error bars showing mean number of resin tags at each section according to group

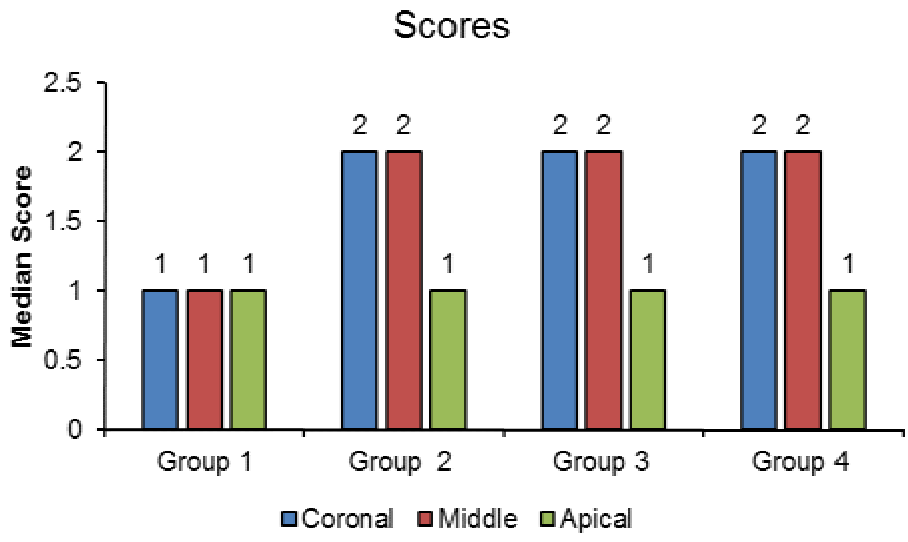

Figure 3: Column chart showing median score at each section according to group

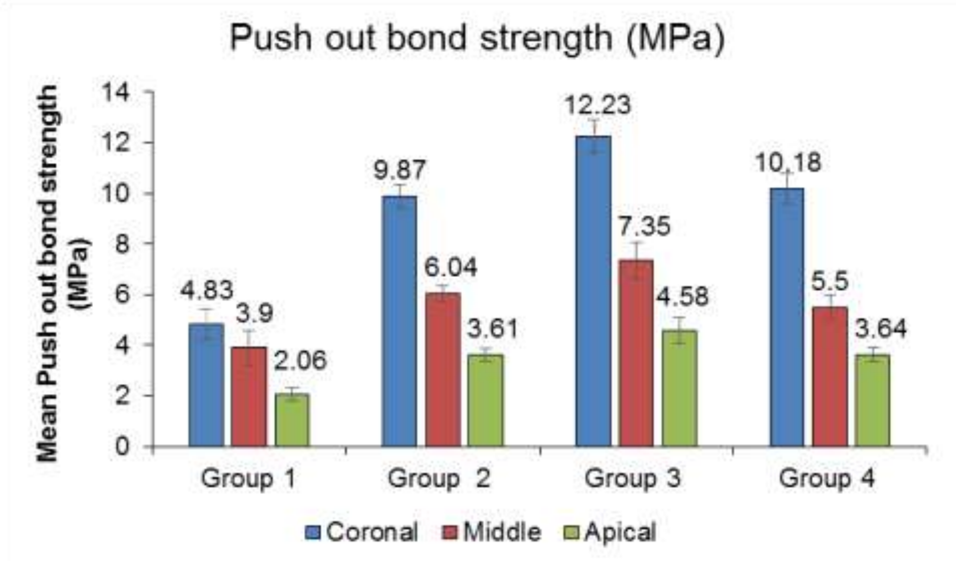

Figure 4: Column chart with error bars showing mean push-out bond strength at each section according to group

ANNEXURE I

Thickness of hybrid layer in Group I for each section.

Group I: Saline			
Sample No.	**Coronal**	**Middle**	**Apical**
	Hybrid layer	**Hybrid layer**	**Hybrid layer**
1	3.15ɥm	3.01ɥm	2.10ɥm
2	2.08ɥm	1.47ɥm	1.76ɥm
3	2.44ɥm	1.55ɥm	1.92ɥm
4	3.28ɥm	2.43ɥm	1.39ɥm
5	2.16ɥm	1.77ɥm	1.55ɥm
6	1.90ɥm	1.63ɥm	2.22ɥm
7	2.08ɥm	2.12ɥm	2.14ɥm
8	3.03ɥm	1.56ɥm	1.34ɥm
9	2.10ɥm	1.67ɥm	2.21ɥm
10	2.22ɥm	2.29ɥm	3.15ɥm
11	1.96ɥm	2.18ɥm	2.03ɥm
12	1.83ɥm	1.50ɥm	2.11ɥm
13	2.26ɥm	3.79ɥm	1.53ɥm
14	3.01ɥm	3.49ɥm	1.22ɥm
15	1.60ɥm	3.02ɥm	1.86ɥm
16	2.50ɥm	2.10ɥm	1.29ɥm
17	2.29ɥm	1.67ɥm	3.00ɥm
18	1.35ɥm	2.00ɥm	1.63ɥm
19	2.12ɥm	3.76ɥm	1.77ɥm
20	1.75ɥm	3.01ɥm	1.47ɥm
21	2.20ɥm	2.16ɥm	2.03ɥm
22	1.64ɥm	2.03ɥm	2.05ɥm
23	2.31ɥm	2.11ɥm	1.86ɥm
24	2.63ɥm	1.90ɥm	2.26ɥm
25	2.45ɥm	1.35ɥm	2.00ɥm

ANNEXURE II

Thickness of hybrid layer in Group II for each section.

Group II: EDTA			
Sample No.	**Coronal**	**Middle**	**Apical**
	Hybrid layer	**Hybrid layer**	**Hybrid layer**
1	5.89ɥm	4.41ɥm	2.50ɥm
2	5.38ɥm	4.98ɥm	3.11ɥm
3	4.98ɥm	4.03ɥm	2.42ɥm
4	5.16ɥm	3.87ɥm	3.67ɥm
5	5.32ɥm	4.05ɥm	3.04ɥm
6	4.71ɥm	3.90ɥm	4.00ɥm
7	5.89ɥm	3.69ɥm	3.59ɥm
8	4.56ɥm	3.26ɥm	3.79ɥm
9	4.07ɥm	4.60ɥm	4.21ɥm
10	5.90ɥm	3.14ɥm	3.12ɥm
11	4.69ɥm	4.96ɥm	3.39ɥm
12	4.04ɥm	3.98ɥm	4.09ɥm
13	5.15ɥm	4.08ɥm	4.37ɥm
14	5.67ɥm	4.76ɥm	4.01ɥm
15	5.56ɥm	3.86ɥm	3.64ɥm
16	4.91ɥm	4.47ɥm	3.49ɥm
17	4.21ɥm	3.60ɥm	4.04ɥm
18	5.24ɥm	3.36ɥm	2.6ɥm
19	3.96ɥm	4.10ɥm	2.23ɥm
20	3.49ɥm	3.18ɥm	3.89ɥm
21	4.86ɥm	4.60ɥm	3.01ɥm
22	4.90ɥm	3.78ɥm	4.23ɥm
23	4.01ɥm	4.03ɥm	2.67ɥm
24	5.69ɥm	4.98ɥm	2.34ɥm
25	4.79ɥm	3.97ɥm	3.36ɥm

ANNEXURE III

Thickness of hybrid layer in Group III for each section.

Group III: Maleic acid			
Sample No.	**Coronal**	**Middle**	**Apical**
	Hybrid layer	**Hybrid layer**	**Hybrid layer**
1	5.54ɥm	6.01ɥm	6.30ɥm
2	5.69ɥm	5.94ɥm	5.89ɥm
3	5.98ɥm	5.11ɥm	5.12ɥm
4	6.01ɥm	5.43ɥm	5.01ɥm
5	6.91ɥm	6.09ɥm	5.44ɥm
6	6.47ɥm	6.49ɥm	5.31ɥm
7	5.65ɥm	5.87ɥm	5.60ɥm
8	6.08ɥm	5.83ɥm	5.03ɥm
9	6.93ɥm	5.11ɥm	5.12ɥm
10	6.13ɥm	5.82ɥm	5.11ɥm
11	6.24ɥm	5.09ɥm	5.71ɥm
12	5.91ɥm	6.00ɥm	5.09ɥm
13	5.85ɥm	6.78ɥm	5.04ɥm
14	5.15ɥm	6.04ɥm	5.00ɥm
15	6.26ɥm	5.01ɥm	6.04ɥm
16	6.19ɥm	5.80ɥm	5.99ɥm
17	5.92ɥm	5.74ɥm	5.55ɥm
18	6.23ɥm	5.04ɥm	5.11ɥm
19	5.87ɥm	5.00ɥm	5.31ɥm
20	5.11ɥm	5.13ɥm	5.21ɥm
21	6.03ɥm	5.89ɥm	6.67ɥm
22	6.22ɥm	5.69ɥm	5.10ɥm
23	6.61ɥm	5.71ɥm	5.13ɥm
24	5.09ɥm	6.03ɥm	5.71ɥm
25	5.32ɥm	5.74ɥm	5.07ɥm

ANNEXURE IV

Thickness of hybrid layer in Group IV for each section.

Group IV: Chitosan			
Sample No.	**Coronal**	**Middle**	**Apical**
	Hybrid layer	**Hybrid layer**	**Hybrid layer**
1	4.67ɥm	5.35ɥm	4.44ɥm
2	5.43ɥm	5.80ɥm	3.32ɥm
3	5.44ɥm	4.19ɥm	4.19ɥm
4	5.98ɥm	4.67ɥm	2.38ɥm
5	5.62ɥm	4.60ɥm	3.71ɥm
6	5.96ɥm	4.26ɥm	4.03ɥm
7	4.59ɥm	3.44ɥm	4.21ɥm
8	4.90ɥm	3.03ɥm	3.22ɥm
9	5.60ɥm	4.11ɥm	2.17ɥm
10	5.21ɥm	4.71ɥm	3.11ɥm
11	5.26ɥm	4.00ɥm	4.05ɥm
12	5.88ɥm	4.01ɥm	4.83ɥm
13	5.35ɥm	3.21ɥm	2.79ɥm
14	5.78ɥm	2.77ɥm	2.07ɥm
15	4.00ɥm	4.72ɥm	4.09ɥm
16	4.99ɥm	4.27ɥm	3.33ɥm
17	5.75ɥm	3.18ɥm	3.04ɥm
18	6.19ɥm	3.14ɥm	2.63ɥm
19	5.99ɥm	3.70ɥm	2.51ɥm
20	4.62ɥm	3.81ɥm	4.91ɥm
21	5.73ɥm	4.70ɥm	3.13ɥm
22	4.37ɥm	3.21ɥm	2.99ɥm
23	5.07ɥm	4.52ɥm	4.10ɥm
24	4.57ɥm	3.02ɥm	5.73ɥm
25	4.71ɥm	4.11ɥm	4.03ɥm

ANNEXURE V

Number of resin tags in Group I for each section.

Observer 1			
GroupI: SALINE			
Sample No.	**Coronal** **No. of resin tags**	**Middle** **No. of resin tags**	**Apical** **No. of resin tags**
1	20	15	11
2	15	19	9
3	23	9	8
4	19	9	6
5	10	9	5
6	19	12	10
7	10	10	8
8	15	7	7
9	12	10	10
10	11	7	8
11	14	12	11
12	23	11	5
13	19	17	11
14	17	16	9
15	16	9	8
16	22	15	4
17	11	11	7
18	12	9	11
19	16	13	10
20	19	8	9
21	14	8	7
22	10	10	4
23	17	11	10
24	13	7	5
25	11	9	4

Observer 2			
Group I: SALINE			
Sample No.	**Coronal** **No. of resin tags**	**Middle** **No. of resin tags**	**Apical** **No. of resin tags**
1	20	17	11
2	13	19	9
3	23	13	8
4	21	9	6
5	10	9	5
6	19	12	12
7	10	10	8
8	15	7	7
9	12	10	10
10	11	9	8
11	14	12	11
12	25	11	5
13	19	17	11
14	17	16	9
15	16	9	8
16	22	15	4
17	15	11	7
18	12	9	11
19	16	13	10
20	19	8	9
21	14	8	7
22	10	10	4
23	13	11	10
24	13	7	5
25	11	9	4

MEAN			
Group I: SALINE			
Sample No.	**Coronal** **No. of resin tags**	**Middle** **No. of resin tags**	**Apical** **No. of resin tags**
1	20	16	11
2	14	19	9
3	23	11	8
4	20	9	6
5	10	9	5
6	19	12	11
7	10	10	8
8	15	7	7
9	12	10	10
10	11	8	8
11	14	12	11
12	24	11	5
13	19	17	11
14	17	16	9
15	16	9	8
16	22	15	4
17	13	11	7
18	12	9	11
19	16	13	10
20	19	8	9
21	14	8	7
22	10	10	4
23	15	11	10
24	13	7	5
25	11	9	4

ANNEXURE VI

Number of resin tags in Group II for each section.

Observer 1			
Group II:EDTA			
Sample No.	Coronal	Middle	Apical
	No. of resin tags	No. of resin tags	No. of resin tags
1	30	21	11
2	28	18	20
3	22	21	10
4	19	20	11
5	17	22	9
6	26	19	20
7	17	26	8
8	21	20	15
9	28	25	17
10	25	20	8
11	19	14	11
12	29	23	5
13	22	21	11
14	21	24	9
15	18	24	8
16	31	25	20
17	26	22	20
18	24	19	22
19	29	17	10
20	32	15	9
21	19	20	7
22	26	18	4
23	22	22	10
24	17	15	5
25	28	23	8

Observer 2			
Group II:EDTA			
Sample No.	Coronal	Middle	Apical
	No. of resin tags	No. of resin tags	No. of resin tags
1	30	23	11
2	28	18	20
3	22	21	10
4	21	20	11
5	17	22	9
6	26	19	20
7	19	26	8
8	21	20	13
9	28	25	17
10	25	20	8
11	19	14	11
12	25	23	5
13	22	21	11
14	25	24	9
15	18	24	8
16	31	25	22
17	26	22	20
18	24	21	18
19	29	17	10
20	32	15	9
21	19	20	7
22	24	18	4
23	22	22	10
24	17	15	5
25	28	19	8

MEAN			
Group II:EDTA			
Sample No.	Coronal	Middle	Apical
	No. of resin tags	No. of resin tags	No. of resin tags
1	30	22	11
2	28	18	20
3	22	21	10
4	20	20	11
5	17	22	9
6	26	19	20
7	18	26	8
8	21	20	14
9	28	25	17
10	25	20	8
11	19	14	11
12	27	23	5
13	22	21	11
14	23	24	9
15	18	24	8
16	31	25	21
17	26	22	20
18	24	20	20
19	29	17	10
20	32	15	9
21	19	20	7
22	25	18	4
23	22	22	10
24	17	15	5
25	28	21	8

ANNEXURE VII

Number of resin tags in Group III for each section

Observer 1			
Group III: Maleic Acid			
Sample No.	Coronal	Middle	Apical
	No. of resin tags	No. of resin tags	No. of resin tags
1	37	20	21
2	24	22	13
3	29	22	19
4	25	24	24
5	20	18	25
6	27	21	18
7	23	19	17
8	23	22	24
9	20	21	15
10	34	24	13
11	30	30	14
12	21	20	13
13	20	21	20
14	27	24	26
15	29	19	24
16	24	17	10
17	30	20	18
18	21	22	15
19	24	29	12
20	21	17	19
21	18	20	14
22	30	20	15
23	27	27	11
24	22	24	22
25	21	20	20

Observer 2			
Group III: Maleic Acid			
Sample No.	Coronal	Middle	Apical
	No. of resin tags	No. of resin tags	No. of resin tags
1	37	20	21
2	24	22	13
3	27	22	19
4	23	24	20
5	20	18	25
6	27	21	18
7	23	19	17
8	23	24	26
9	20	21	15
10	34	24	13
11	34	32	14
12	21	20	13
13	20	21	22
14	23	26	26
15	29	19	24
16	24	17	10
17	30	20	18
18	25	22	15
19	24	29	12
20	21	17	21
21	18	20	14
22	28	20	15
23	27	25	11
24	22	24	22
25	25	20	20

MEAN			
Group III: Maleic Acid			
Sample No.	Coronal	Middle	Apical
	No. of resin tags	No. of resin tags	No. of resin tags
1	37	20	21
2	24	22	13
3	28	22	19
4	24	24	22
5	20	18	25
6	27	21	18
7	23	19	17
8	23	23	25
9	20	21	15
10	34	24	13
11	32	31	14
12	21	20	13
13	20	21	21
14	25	25	26
15	29	19	24
16	24	17	10
17	30	20	18
18	23	22	15
19	24	29	12
20	21	17	20
21	18	20	14
22	29	20	15
23	27	26	11
24	22	24	22
25	23	20	20

ANNEXURE VIII

Number of resin tags in Group IV for each section.

Observer 1			
Group IV:Chitosan			
Sample No.	Coronal No. of resin tags	Middle No. of resin tags	Apical No. of resin tags
1	27	24	10
2	25	22	21
3	29	22	9
4	20	17	12
5	18	22	10
6	32	20	20
7	23	17	7
8	21	22	12
9	21	26	16
10	29	19	9
11	29	25	12
12	24	22	7
13	21	19	9
14	29	26	8
15	22	17	8
16	21	18	20
17	25	22	19
18	21	20	22
19	25	25	10
20	20	15	8
21	17	17	7
22	25	18	5
23	22	20	9
24	20	12	6
25	20	15	12

Observer 2			
Group IV: Chitosan			
Sample No.	Coronal No. of resin tags	Middle No. of resin tags	Apical No. of resin tags
1	27	24	10
2	23	22	21
3	29	22	9
4	20	21	12
5	20	22	10
6	32	20	22
7	23	17	7
8	21	22	12
9	21	22	16
10	29	19	9
11	33	25	12
12	24	24	7
13	21	19	9
14	27	26	8
15	22	19	8
16	21	18	20
17	25	22	21
18	21	20	22
19	25	25	10
20	20	15	8
21	17	17	7
22	25	18	5
23	22	20	9
24	20	12	6
25	20	15	12

MEAN			
Group IV:Chitosan			
Sample No.	Coronal No. of resin tags	Middle No. of resin tags	Apical No. of resin tags
1	27	24	10
2	24	22	21
3	29	22	9
4	20	19	12
5	19	22	10
6	32	20	21
7	23	17	7
8	21	22	12
9	21	24	16
10	29	19	9
11	31	25	12
12	24	23	7
13	21	19	9
14	28	26	8
15	22	18	8
16	21	18	20
17	25	22	20
18	21	20	22
19	25	25	10
20	20	15	8
21	17	17	7
22	25	18	5
23	22	20	9
24	20	12	6
25	20	15	12

ANNEXURE IX

Scoring of resin tags Group I for each section

Observer 1				Observer 2				Observer 3				FINAL SCORE			
Group I: SALINE				Group I: SALINE				Group I: SALINE				Group I: SALINE			
Sample No.	Coronal Score	Middle score	Apical score	Sample No.	Coronal Score	Middle score	Apical score	Sample No.	Coronal Score	Middle score	Apical score	Sample No.	Coronal Score	Middle score	Apical score
1	2	2	1	**1**	2	1	1	**1**	_	1	_	**1**	2	1	1
2	1	1	1	**2**	1	1	1	**2**	_	_	_	**2**	1	1	1
3	2	1	1	**3**	2	1	1	**3**	_	_	_	**3**	2	1	1
4	1	1	1	**4**	2	1	1	**4**	2	_	_	**4**	2	1	1
5	1	1	1	**5**	1	1	1	**5**	_	_	_	**5**	1	1	1
6	2	1	2	**6**	2	1	1	**6**	_	_	1	**6**	2	1	1
7	2	1	1	**7**	2	1	1	**7**	_	_	_	**7**	2	1	1
8	2	1	1	**8**	2	1	1	**8**	_	_	_	**8**	2	1	1
9	1	1	1	**9**	1	1	1	**9**	_	_	_	**9**	1	1	1
10	1	2	0	**10**	1	1	0	**10**	_	1	_	**10**	1	1	0
11	1	2	1	**11**	1	2	1	**11**	_	_	_	**11**	1	2	1
12	2	1	0	**12**	1	1	0	**12**	1	_	_	**12**	1	1	0
13	1	2	1	**13**	1	2	1	**13**	_	_	_	**13**	1	2	1
14	1	1	1	**14**	1	1	1	**14**	_	_	_	**14**	1	1	1
15	2	1	0	**15**	2	1	0	**15**	_	_	_	**15**	2	1	0
16	1	1	1	**16**	1	1	1	**16**	_	_	_	**16**	1	1	1
17	2	1	1	**17**	1	1	1	**17**	1	_	_	**17**	1	1	1
18	1	1	1	**18**	1	1	1	**18**	_	_	_	**18**	1	1	1
19	2	1	1	**19**	2	1	1	**19**	_	_	_	**19**	2	1	1
20	1	1	1	**20**	1	1	1	**20**	_	_	_	**20**	1	1	1
21	1	2	1	**21**	1	2	1	**21**	_	_	_	**21**	1	2	1
22	2	1	1	**22**	2	1	0	**22**	_	_	0	**22**	2	1	0
23	1	1	1	**23**	1	1	1	**23**	_	_	_	**23**	1	1	1
24	1	1	1	**24**	1	1	1	**24**	_	_	_	**24**	1	1	1
25	1	1	0	**25**	2	1	0	**25**	1	_	_	**25**	1	1	0

ANNEXURE X

Scoring of resin tags Group II for each section

Observer 1			
Group II: EDTA			
Sample No.	Coronal Score	Middle score	Apical score
1	2	2	1
2	3	2	2
3	2	2	1
4	2	2	1
5	1	2	1
6	2	1	2
7	1	3	1
8	2	2	2
9	3	3	2
10	2	2	1
11	2	2	2
12	2	2	1
13	2	3	1
14	2	2	2
15	2	2	1
16	3	2	2
17	2	2	2
18	2	3	2
19	3	1	1
20	3	2	1
21	2	2	2
22	2	2	2
23	2	2	1
24	2	1	2
25	3	2	1

Observer 2			
Group II: EDTA			
Sample No.	Coronal Score	Middle score	Apical score
1	2	1	1
2	3	2	2
3	2	2	1
4	2	2	1
5	1	2	1
6	2	1	2
7	2	3	1
8	2	2	2
9	3	3	2
10	2	2	1
11	2	2	2
12	3	2	1
13	2	3	1
14	1	2	2
15	2	2	1
16	3	2	1
17	2	2	2
18	2	2	2
19	3	1	1
20	3	2	1
21	2	2	2
22	3	2	2
23	2	2	1
24	2	1	2
25	3	1	1

Observer 3			
Group II: EDTA			
Sample No.	Coronal Score	Middle score	Apical score
1	_	2	_
2	_	_	_
3	_	_	_
4	_	_	_
5	_	_	_
6	_	_	_
7	1	_	_
8	_	_	_
9	_	_	_
10	_	_	_
11	_	_	_
12	3	_	_
13	_	_	_
14	2	_	_
15	_	_	_
16	_	_	2
17	_	_	_
18	_	2	_
19	_	_	_
20	_	_	_
21	_	_	_
22	3	_	_
23	_	_	_
24	_	_	_
25	_	2	_

FINAL SCORE			
Group II: EDTA			
Sample No.	Coronal Score	Middle score	Apical score
1	2	2	1
2	3	2	2
3	2	2	1
4	2	2	1
5	1	2	1
6	2	1	2
7	1	3	1
8	2	2	2
9	3	3	2
10	2	2	1
11	2	2	2
12	3	2	1
13	2	3	1
14	2	2	2
15	2	2	1
16	3	2	2
17	2	2	2
18	2	2	2
19	3	1	1
20	3	2	1
21	2	2	2
22	3	2	2
23	2	2	1
24	2	1	2
25	3	2	1

ANNEXURE XI

Scoring of resin tags Group III for each section

Observer 1				Observer 2				Observer 3				FINAL SCORE			
Group III: Maleic acid				Group III: Maleic acid				Group III: Maleic acid				Group III: Maleic acid			
Sample No.	Coronal Score	Middle score	Apical score	Sample No.	Coronal Score	Middle score	Apical score	Sample No.	Coronal Score	Middle score	Apical score	Sample No.	Coronal Score	Middle score	Apical score
1	3	2	2	**1**	3	2	2	**1**	__	__	__	**1**	3	2	2
2	2	2	1	**2**	2	2	1	**2**	__	__	__	**2**	2	2	1
3	3	2	1	**3**	2	2	1	**3**	3	__	__	**3**	3	2	1
4	2	2	2	**4**	3	2	1	**4**	2	__	2	**4**	2	2	2
5	2	1	3	**5**	2	1	3	**5**	__	__	__	**5**	2	1	3
6	3	2	2	**6**	3	2	2	**6**	__	__	__	**6**	3	2	2
7	2	2	1	**7**	2	2	1	**7**	__	__	__	**7**	2	2	1
8	2	2	3	**8**	2	3	3	**8**	__	2	__	**8**	2	2	3
9	2	2	1	**9**	2	2	1	**9**	__	__	__	**9**	2	2	1
10	3	2	1	**10**	3	2	1	**10**	__	__	__	**10**	3	2	1
11	3	2	2	**11**	3	3	2	**11**	__	3	__	**11**	3	3	2
12	2	2	1	**12**	2	2	1	**12**	__	__	__	**12**	2	2	1
13	2	2	2	**13**	2	2	3	**13**	__	__	2	**13**	2	2	2
14	3	3	3	**14**	2	2	3	**14**	3	3	__	**14**	3	3	3
15	3	1	2	**15**	3	1	2	**15**	__	__	__	**15**	3	1	2
16	2	1	1	**16**	2	1	1	**16**	__	__	__	**16**	2	1	1
17	3	2	2	**17**	3	2	2	**17**	__	__	__	**17**	3	2	2
18	3	2	2	**18**	2	2	2	**18**	2	__	__	**18**	2	2	2
19	3	3	1	**19**	3	3	1	**19**	__	__	__	**19**	3	3	1
20	2	1	1	**20**	2	1	2	**20**	__	__	2	**20**	2	1	2
21	3	2	1	**21**	3	2	1	**21**	__	__	__	**21**	3	2	1
22	2	2	1	**22**	3	2	1	**22**	3	__	__	**22**	3	2	1
23	2	2	2	**23**	2	3	2	**23**	__	3	__	**23**	2	3	2
24	2	2	2	**24**	2	2	2	**24**	__	__	__	**24**	2	2	2
25	2	2	2	**25**	3	2	2	**25**	2	__	__	**25**	2	2	2

ANNEXURE XII

Scoring of resin tags Group IV for each section

Observer 1			
Group IV: Chitosan			
Sample No.	Coronal Score	Middle score	Apical score
1	3	2	1
2	2	2	2
3	3	2	1
4	2	2	2
5	1	2	1
6	3	3	1
7	2	1	1
8	2	2	2
9	2	3	1
10	3	1	1
11	2	3	2
12	2	2	1
13	2	1	1
14	3	3	1
15	2	1	1
16	2	2	2
17	3	2	2
18	2	3	2
19	3	3	1
20	2	1	1
21	1	1	1
22	3	1	1
23	2	2	1
24	2	1	1
25	2	1	1

Observer 2			
Group IV: Chitosan			
Sample No.	Coronal Score	Middle score	Apical score
1	3	2	1
2	3	2	2
3	3	2	1
4	2	2	2
5	2	2	1
6	3	3	2
7	2	1	1
8	2	2	2
9	2	2	1
10	3	1	1
11	3	3	2
12	2	2	1
13	2	1	1
14	2	3	1
15	2	2	1
16	2	2	2
17	3	2	1
18	2	3	2
19	3	3	1
20	2	1	1
21	1	1	1
22	3	1	1
23	2	2	1
24	2	1	1
25	2	1	1

Observer 3			
Group IV: Chitosan			
Sample No.	Coronal Score	Middle score	Apical score
1	_	_	_
2	2	_	_
3	_	_	_
4	_	_	_
5	1	_	_
6	_	_	2
7	_	_	_
8	_	_	_
9	_	2	_
10	_	_	_
11	3	_	_
12	_	_	_
13	_	_	_
14	3	_	_
15	_	1	_
16	_	_	_
17	_	_	2
18	_	_	_
19	_	_	_
20	_	_	_
21	_	_	_
22	_	_	_
23	_	_	_
24	_	_	_
25	_	_	_

FINAL SCORE			
Group IV: Chitosan			
Sample No.	Coronal Score	Middle score	Apical score
1	3	2	1
2	2	2	2
3	3	2	1
4	2	2	2
5	1	2	1
6	3	3	2
7	2	1	1
8	2	2	2
9	2	2	1
10	3	1	1
11	3	3	2
12	2	2	1
13	2	1	1
14	3	3	1
15	2	1	1
16	2	2	2
17	3	2	2
18	2	3	2
19	3	3	1
20	2	1	1
21	1	1	1
22	3	1	1
23	2	2	1
24	2	1	1
25	2	1	1

ANNEXURE XIII

Push-Out bond strength of fiber post to root dentin in Group I for each section

Group I: SALINE						
Sample 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	21.5	2.86	46	6.66	13.5	2.15
2	20	2.66	43	6.22	19.5	3.1
3	49.5	6.57	39	5.64	9	1.43
4	63	8.37	41	5.93	11	1.75
5	39	5.18	22	3.18	17.5	2.79
6	53	7.03	27	3.9	7.5	1.19
7	47	6.24	19	2.74	14	2.23
8	49	6.5	18	2.6	18.5	2.95
9	51	6.77	12	1.74	10	1.59
10	37	4.91	19.5	2.82	12.5	1.99
11	25	3.32	36	5.2	13	2.07
12	42	5.58	39	5.64	8.5	1.35
13	27.5	3.65	14.5	2.09	14.5	2.31
14	21	2.79	28	4.05	19	3.02
15	24	3.19	17.5	2.53	13.5	2.15
16	31	4.12	38	5.5	5.5	0.87
17	36	4.78	18.5	2.68	21.5	3.4
18	37.5	4.98	20	2.89	13.5	2.15
19	27	3.59	9	1.3	10.5	1.67
20	24.5	3.25	21.5	3.11	11.5	1.83
21	43	5.71	49	7.09	16	2.54
22	33	4.38	40	5.79	15.5	2.46
23	28	3.72	16	2.32	12	1.91
24	38.5	5.11	25	3.61	8.5	1.35
25	41.5	5.51	15.5	2.24	8	1.27

ANNEXURE XIV

Push-Out bond strength of fiber post to root dentin in Group II for each section

Group II: EDTA						
Sample 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	75	9.96	40.5	5.86	19	3.02
2	68	9.03	39.5	5.72	22	3.5
3	81	10.76	36.5	5.28	27.5	4.38
4	73.5	9.76	42.5	6.15	30.5	4.86
5	74.5	9.89	47.5	6.87	21	3.34
6	97.5	12.95	43.5	6.3	17.5	2.79
7	89	11.82	38	5.5	18.5	2.95
8	70.5	9.36	49.5	7.16	28	4.46
9	87.5	11.62	50	7.24	19.5	3.1
10	63	8.37	48.5	7.01	27	4.3
11	72	9.56	46	6.66	17	2.71
12	69.5	9.23	45.5	6.58	22.5	3.58
13	71.5	9.5	41	5.93	21.5	3.4
14	82.5	10.96	43	6.22	23.5	3.74
15	65.5	8.7	44.5	6.44	24	3.82
16	73	9.69	34.5	4.99	27.5	4.38
17	74	9.83	32	4.63	22	3.5
18	67.5	8.96	39	5.64	26.5	4.21
19	83.5	11.09	33.5	4.85	28.5	4.54
20	79.5	10.56	46.5	6.73	18	2.87
21	75	9.96	44	6.37	21	3.34
22	62	8.23	41.5	6.01	26	4.14
23	70	9.29	35.5	5.14	20.5	3.26
24	60.5	8.03	42	6.08	18.5	2.95
25	72.5	9.63	39	5.64	20	3.18

ANNEXURE XV

Push-Out bond strength of fiber post to root dentin in Group III for each section

Group III: Maleic Acid						
Sample 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	110	14.61	73	10.56	27.5	4.38
2	98	13.01	51	7.38	46	7.32
3	82.5	10.96	63	9.12	43.5	6.93
4	105	13.94	41	5.93	39	6.29
5	101.5	13.52	47	6.8	41.5	6.61
6	81	10.76	42	6.08	23	3.66
7	110	14.61	87.5	12.66	22	3.5
8	107.5	14.28	49	7.09	27.5	4.38
9	87.5	11.62	53	7.67	19.5	3.11
10	95	12.62	45.5	6.58	36	5.73
11	87.5	11.62	49.5	7.09	30.5	4.86
12	79.5	10.56	72	10.42	39	6.21
13	104.5	13.88	56.5	8.18	21	3.34
14	92.5	12.28	50.5	7.31	24	3.82
15	83	11.02	58	8.39	19.5	3.11
16	105.5	14.01	49	7.09	27	4.3
17	96.5	12.81	37.5	5.35	30	4.78
18	72	9.56	44	6.37	26	4.14
19	101	13.41	51.5	7.38	21.5	3.42
20	81.5	10.82	47.5	6.87	34	5.41
21	70.5	9.36	40	5.79	22.5	3.58
22	81	10.76	36.5	5.28	28	4.46
23	73	9.69	33.5	4.85	20	3.18
24	97	12.88	38.5	5.57	26.5	4.22
25	100	13.28	54.5	7.89	23	3.67

ANNEXURE XVI

Push-Out bond strength of fiber post to root dentin in Group IV for each section

Group IV: Chitosan						
Sample 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	89	11.82	39	5.64	21.5	3.42
2	97.5	12.95	44	6.37	27.5	4.38
3	73.5	9.76	37.5	5.35	22	3.05
4	74.5	9.89	33.5	4.85	19	3.02
5	81	10.76	27.5	3.98	18.5	2.94
6	75	9.96	30	4.34	19.5	3.11
7	68	9.03	34.5	4.99	21	3.34
8	70.5	9.36	40.5	5.86	27	4.3
9	63	8.37	36.5	5.28	17	2.71
10	81	10.76	32	4.63	23.5	3.74
11	73	9.69	38.5	5.57	21.5	3.42
12	87.5	11.62	42.5	6.15	29	4.62
13	98	13.01	55	7.96	23	3.66
14	82.5	10.96	39.5	5.72	18	2.87
15	72	9.56	31.5	4.56	17.5	2.79
16	77.5	10.29	29	4.2	26.5	4.22
17	92.5	12.28	53	7.67	26	4.14
18	59.5	7.9	34.5	4.99	30	4.78
19	67.5	8.96	43	6.22	28.5	4.54
20	98	13.01	47	6.8	16.5	2.63
21	78.5	10.42	54.5	7.89	23.5	3.74
22	62	8.23	28	4.05	24	3.82
23	57.5	7.64	23.5	3.4	19.5	3.11
24	70.5	9.36	35.5	5.14	26	4.14
25	68	9.03	41	5.93	28	4.46