

**A CONFOCAL LASER SCANNING MICROSCOPIC
EVALUATION OF THE HYBRID LAYER AND RESIN TAGS
AT RESIN- DENTIN INTERFACE IN THE ADHESIVE RESIN
LUTING SYSTEMS AND THE EFFECTS OF ACTIVE AND
PASSIVE IRRIGATION ON THEM - AN IN VITRO STUDY**

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CONTENTS



SR.NO	TITLES	PAGE NO.
01	INTRODUCTION	1-5
02	AIM AND OBJECTIVES	6-7
03	REVIEW OF LITERATURE	8-21
04	MATERIALS AND METHOD	22-31
05	RESULTS	32-41
06	DISCUSSION	42-57
07	LIMITATIONS	58
08	SUMMARY AND CONCLUSION	59-61
09	BIBLIOGRAPHY	62-73
10	TABLES AND GRAPHS	i-xvi
11	ANNEXURE	xvii-xxii

LIST OF TABLES



SR. NO	TITLES
Table 1:	Descriptive Statistics i.e Mean and Standard Deviation for thickness of hybrid layer according to groups (Active Irrigation and Passive Irrigation) and subgroups (Clearfill SE, Panavia, Prime and bond NT) at Coronal, Middle and Apical sections.
Table 2:	Comparison between groups and subgroups and their effect on thickness of hybrid layer at CORONAL section using two-way analysis of variance
Table 2a:	Comparison of main effect of Irrigation Group on thickness of hybrid layer at CORONAL section as per subgroups
Table 2b:	Comparison of main effect of subgroups on thickness of hybrid layer at CORONAL section as per groups
Table 3:	Comparison between groups and subgroups and their effect on thickness of hybrid layer at MIDDLE section using two-way analysis of variance
Table 3a:	Descriptive statistics for thickness of hybrid layer at group level for MIDDLE section
Table 3b:	Descriptive statistics for thickness of hybrid layer at subgroup level for MIDDLE section
Table 3c:	Paired comparison of thickness of hybrid layer between subgroups for MIDDLE section using Tukey's post-hoc test
Table 4:	Comparison between groups and subgroups and their effect on thickness of hybrid layer at APICAL section using two-way analysis of variance.
Table 4a:	Descriptive statistics for thickness of hybrid layer at group level for APICAL section

SR. NO	TITLES
Table 4b:	Descriptive statistics for thickness of hybrid layer at subgroup level for APICAL section
Table 4c:	Paired comparison of thickness of hybrid layer between subgroups for APICAL section using Tukey's post-hoc test
Table 5:	Descriptive Statistics i.e Mean and Standard Deviation for no. of resin tags according to groups (Active Irrigation and Passive Irrigation) and subgroups (Clearfill SE, Panavia, Prime and bond NT) at Coronal, Middle and Apical sections.
Table 6:	Comparison between groups and subgroups and their effect on no. of resin tags at CORONAL section using two-way analysis of variance
Table 6a:	Descriptive statistics for no. of resin tags at group level for CORONAL section
Table 6b:	Descriptive statistics for no. of resin tags at sub group level for CORONAL section
Table 6c:	Paired comparison of no. of resin tags between subgroups for CORONAL section using Tukey's post-hoc test
Table 7:	Comparison between groups and subgroups and their effect on no. of resin tags at MIDDLE section using two-way analysis of variance
Table 7a:	Descriptive statistics for no. of resin tags at group level for MIDDLE section
Table 7b:	Descriptive statistics for no. of resin tags at subgroup level for MIDDLE section
Table 7c:	Paired comparison of no. of resin tags between subgroups for MIDDLE section using Tukey's post-hoc test
Table 8:	Comparison between groups and subgroups and their effect on no. of resin tags at APICAL section using two-way analysis of variance
Table 8a:	Comparison of main effect of Irrigation Group on no. of resin tags at APICAL section as per subgroups
Table 8b:	Table 8b: Comparison of main effect of subgroups on no. of resin tags at APICAL section as per groups

LIST OF GRAPHS



SR. NO	TITLES
Figure 1:	Bar chart with error bars showing mean thickness of hybrid layer according to groups and subgroups for CORONAL section
Figure 2:	Bar chart with error bars showing mean thickness of hybrid layer according to groups and subgroups for MIDDLE section
Figure 3:	Bar chart with error bars showing mean thickness of hybrid layer according to groups and subgroups for APICAL section
Figure 4:	Mean thickness of hybrid layer for each subgroups according to groups for CORONAL section
Figure 5:	Mean thickness of hybrid layer for each group according to subgroups for CORONAL section
Figure 6:	Mean thickness of hybrid layer for each group according to subgroups for MIDDLE section
Figure 7:	Mean thickness of hybrid layer for each group according to subgroups for APICAL section
Figure 8:	Bar chart with error bars showing mean number of resin tags according to groups and subgroups for CORONAL section
Figure 9:	Bar chart with error bars showing mean number of resin tags according to groups and subgroups for MIDDLE section
Figure 10:	Bar chart with error bars showing mean number of resin tags according to groups and subgroups for APICAL section
Figure 11:	Mean number of resin tags for each group according to subgroups for CORONAL section

SR. NO	TITLES
Figure 12:	Mean number of resin tags for each group according to subgroups for MIDDLE section
Figure 13:	Mean number of resin tags for each sub group according to groups for APICAL section

LIST OF COLOUR PLATES



SR.NO	TITLE	PLATE NO.
01.	Hand instruments (GDC, India)	I
02.	Cotton Holder & Waste receiver (GDC, India)	
03.	Digital Vernier Caliper (Workzone tools, Germany)	
04.	Mixing pad, Spatula, Microbrushes, Dispenser	
05.	Straight handpiece, Double Sided Diamond Disc	II
06.	Endodontic motor X-Smart (Dentsply, Maillefer, Ballaigues, Switzerland)	
07.	Gates Glidden Drills (Mani, Japan)	
08.	Endobloc (Dentsply, Maillefer, Ballaigues, Switzerland)	
09.	Standard 2% K and H files #15-80 (Mani, Japan)	III
10.	Reamers (Mani, Japan)	
11.	Pluggers and Spreader (Mani, Japan)	
12.	LentuloSpiral (Mani, Japan)	
13.	Peeso Drills (Mani, Japan)	
14.	Hot tip (Discus Dental Inc, USA)	IV
15.	Endoactivator (Dentsply, Maillefer, Ballaigues, Switzerland)	
16.	LED Light Curing Gun (3M ESPE, Germany)	
17.	L-Mould	
18.	Grinder and Polisher (Buehler, Germany)	
19.	Sodium Hypochlorite (Hyposept UPS Hygienes, India)	V
20.	0.9% w/v Normal Saline (Nirlife, India)	
21.	Dent Wash 17% Liquid EDTA (Prime Dental Products, India)	
22.	Irrigation Syringe and Needle (Nirlife, India)	
23.	RC Help 17% EDTA Chelating gel (Prime Dental Products, India)	
24.	Absorbant Points (DiaDent, Korea)	
25.	Gutta Percha Points (DiaDent, Korea)	

SR.NO	TITLE	PLATE NO.
26.	AH Plus Resin Sealer (Dentsply, Maillefer, Ballaigues, Switzerland)	VI
27.	Cavit G (3M ESPE, Germany)	
28.	Rhodamine B Dye (Loba chemie, India)	
29.	Fibre Post (Angelus, Brazil)	
30.	Auto polymerized Acrylic Resin (DPI-RR Cold Cure)	
31.	Clearfill (Kuraray Noritake, Japan)	VII
32.	Panavia (Kuraray Noritake, Japan)	
33.	Prime and Bond NT, Calibra Resin Cement (Dentsply, Maillefer, Ballaigues, Switzerland)	
34.	Sample size (N=120)	VIII
35.	Pre Operative Radiographs	
36.	Decoronation Of Samples	
37.	Decoronated Samples (n=20)	
38.	Length Measured with Digital Vernier Caliper	
39.	Coronal Enlargement with Gates Glidden Drill	IX
40.	Working Length Determination	
41.	Biomechanical preparation	
42.	Irrigation of Root Canal	
43.	Master Cone Selection	
44.	Radiograph of Obturated Root Canal	X
45.	Post Space Preparation	
46.	Group I: Post Space Active Irrigation	
47.	Group II: Post Space Passive Irrigation	
48.	Trial of Fibre Post	XI
49.	Dispensing and Mixing of Primer I and II	
50.	Labelling with Rhodamine B Dye	
51.	Application of Primer with Microbrush	
52.	Manipulation of Luting Cement	
53.	Application of Luting Cement with Lentulo Drill	
54.	Seating of Fibre Post and Curing	

SR.NO	TITLE		PLATE NO.
55.	Sectioning of samples with Isomet Precision Saw		XII
56.	Polishing of samples on Grinder and Polisher		
57.	Sectioned Samples		
58.	Confocal Laser Scanning Microscope (LSM 510, ZIESS with LSM Software ZEN 2007)		
59.	Confocal Laser Scanning Microscopic Images: Group A		XIII
	Group I Active Irrigation	Group II Passive Irrigation	
	Coronal	Coronal	
	Middle	Middle	
	Apical	Apical	
60.	Confocal Laser Scanning Microscopic Images: Group B		XIV
	Group I Active Irrigation	Group II Passive Irrigation	
	Coronal	Coronal	
	Middle	Middle	
	Apical	Apical	
61.	Confocal Laser Scanning Microscopic Images: Group C		XV
	Group I Active Irrigation	Group II Passive Irrigation	
	Coronal	Coronal	
	Middle	Middle	
	Apical	Apical	

LIST OF ABBREVIATIONS



Sr. no	Abbreviations	Full form
01.	C-Factor	Configuration Factor
02.	SEM	Scanning Electron Microscope
03.	TEM	Transmission Electron Microscope
04.	CLSM	Confocal Laser Scanning Microscope
05.	SE	Self Etch
06.	EDTA	Ethylene Diamine Tetra-acetic Acid
07.	NaOCl	Sodium Hypochlorite
08.	RDIZ	Resin Dentin Interdiffusion Zone
09.	min	Minutes
10.	mL	Milli litres
11.	Ni-Ti	Nickle Titanium
12.	CLC	Cold Lateral Condensation
13.	WVC	Warm Vertical Condensation
14.	RITC	Rhodamine B IsoThioCynate
15.	NaCl	Sodium Chloride
16.	HL	Hybrid Layer
17.	RT	Resin Tags
18.	PUI	Passive Ultrasonic Irrigation
19.	OSHA	Occupational Safety and Health Administration
20.	CDC	Centre for Disease Control
21.	LED	Light Emitting Diode
22.	RVG	Radio Visio Graphy
23.	CEJ	Cemento-Enamel Junction
24.	WL	Working Length

Sr. no	Abbreviations	Full form
25.	s	Seconds
26.	Ar	Argon
27.	Kr	Krypton
28	SPSS	Statistical Package for the Social Sciences
29.	µm	Micro meter
30.	ANOVA	Analysis of Variance
31.	HSD	Honest Significant Difference
32.	SD	Standard Deviation
33.	S	Significant
34.	NS	Not Significant
35.	HS	Highly Significant
36.	N	Number of specimens
37.	p-value	Probability of obtaining a test statistic at least as extreme as the one that was actually observed
38.	Max.	Maximum
39.	Min.	Minimum
40.	No.	Number
41.	CI	Confidence Interval
42.	CHX	Chlorhexidine
43.	Bis-GMA	Bisphenol A Glycidil Methacrylate
44.	NT	Nano Technology
45.	mm	Milli Meter

INTRODUCTION

INTRODUCTION

“The best plan for success is to begin with the end in mind.”

Endodontic therapy is considered complete and successful only after the final placement of a permanent, definitive, post-endodontic restoration.¹ Restorative treatment of endodontically treated teeth may vary, ranging from a relatively small direct restoration to more complex indirect restorations involving the placement of an intraradicular post and core and the indirect restoration itself.²

Definitive restoration of endodontically treated teeth aim not only to promote coronal sealing and avoid microleakage/contamination, but also to replace the lost tooth structure and protect the remnant tooth structure, mainly against fractures.³

Teeth exhibiting little or no coronal remnant often require the use of intraradicular post and core to retain the coronal restoration.⁴ Traditionally, posts were always metallic – either cast gold alloy or prefabricated stainless steel or titanium alloy. In the past two decades, non-metallic posts have been introduced including carbon fibre posts (black) and more recently, glass fibre posts, composite posts (white) and zirconium posts (white).

Fiber posts bonded to root canal dentin via resin cements are routinely employed for the restoration of endodontically treated teeth. The similarity in elastic moduli of the fiber post, resin cement, core material and dentin was perceived to be advantageous in improving the performance of these restorations, as compared with cast metal post and core restorations.^{5,6}

Adaptation of adhesive systems for fibre post bonding in root canal is an attractive clinical concept, but its implementation is controversial for several reasons⁷⁻¹¹: influence of the endodontic procedure, polymerization shrinkage (**Feilzer et al. 1993**⁷, **Carvalho et al. 1996**⁸), unfavourable cavity configuration factor (C-Factor) (**Tay et al. 2005**⁹), poor control of moisture (**Bouillaguet et al. 2003**¹⁰) or polymerization difficulties in the apical regions (**Roberts et al. 2004**¹¹).

The bonding principle of dental adhesives is based on the formation of a hybrid layer (**Nakabayashi et al. 1991**¹²) as well as the penetration of adhesive into dentinal

tubules and the formation of ‘resin tags’ (**Titley et al. 1995**¹³, **Ferrari & Davidson 1996**¹⁴). However, the importance of the resin tags and hybrid layer in the quality of the bond is still unclear.

Pegoretti et al (2002) noted that the resultant homogeneous biomechanical unit allows a more uniform stress distribution, which better preserves the weakened tooth structure and reduces microleakage at the dentin-cement interface as well as reinfection of the periapical area.¹⁵

The coupling of resin-based cements requires the adjunctive use of dentin adhesives. Various types of adhesives systems that either follow a ‘total etch’ or ‘self-etch’ approach have been used to bond fibre posts into root canals. Total-etch resin cements utilize phosphoric acid etching that completely dissolves the smear layer and creates a zone of partially demineralized dentin. Upon rinsing the acid conditioners, adhesive primers and resins are applied to the demineralized dentin to achieve micromechanical bonding. Conversely, self-etch resin cements utilize adhesives containing increased concentrations of acidic resin monomers to simultaneously demineralize and infiltrate the smear layer covered dentin.

Although bonding to the root dentin wall has made undeniable progress in recent years, the loss of adhesion at the adhesive/root dentin interface is still the main reason for leakage (**Ferrari & Davidson 1996**¹⁴), decrease in bond strength (**Bouillaguet et al. 2003**¹⁰) and hence, failure of restorations (**Ferrari et al. 2000**¹⁶, **Bouillaguet et al. 2003**¹⁰, **Mannocci et al. 2003**¹⁷).

Also, an observational clinical study of endodontically treated teeth restored with adhesively-luted fiber posts demonstrated an annual failure rate of 4.6% after ten years. The most frequently occurring failure modes were post debonding and post fracture.¹⁸ **Bitter et al** concluded that the achievement of reliable bonding and effective adhesion inside the root canal is a determinant factor in survival of endodontically treated teeth with post and core restorations. Thus, effective bonding between post, dentin, and adhesive resin cement and its durability are essential for the longevity of the restorations.¹⁹

Previous research has suggested that the efficacy of the dentin adhesives mostly depends on the smear layer removal and the resin dentin interdiffusion zone formation. Radicular dentin surfaces are always covered with a thick smear layer and debris after mechanical preparation, which might prevent effective resin bonding.²⁰ It is difficult to remove the smear layer in the root canal with conventional syringe irrigation i.e passive irrigation because of the narrow and deep circumstance of post space. Active irrigation procedures have been used for post space irrigation but their effect on resin dentin interdiffusion zone has not been vastly studied.

Adhesion testing involves qualitative and quantitative assessments. In the investigation of qualitative aspects of bonding, microscopic techniques for high-resolution imaging of interfaces, such as scanning electron microscopy (SEM) and transmission electron microscopy (TEM), confocal laser scanning microscope (CLSM) have found the applications. CLSM offers several advantages including the ability to control depth of field, elimination or reduction of background information away from

the focal plane, and the capability to collect serial optical sections from thick specimens.²¹

Thus, the aim of this in vitro study was to evaluate the hybrid layer and resin tags at resin dentin interface in the adhesive resin luting systems and the effect of active and passive irrigation on them using Confocal Laser Scanning Microscopy (CLSM).

The nulls hypothesis was that there is no difference in the hybrid layer thickness and no. of resin tags at the resin dentin interface in the adhesive resin luting systems when used with active or passive irrigation.

AIM AND OBJECTIVES

AIM & OBJECTIVES

AIM:

To evaluate the thickness of hybrid layer and no. of resin tags at resin- dentin interface in the adhesive resin luting systems and the effect of active and passive irrigation on them by confocal laser scanning microscope.

OBJECTIVES:

1. To evaluate the thickness of hybrid layer and no. of resin tags for the samples passively irrigated and bonded using Clearfill SE, Panavia, Prime & Bond.
2. To evaluate the thickness of hybrid layer and no. of resin tags for the samples actively irrigated and bonded using Clearfill SE, Panavia, Prime & Bond.

3. To compare and evaluate the thickness of hybrid layer and no. of resin tags for the samples passively irrigated and bonded using Clearfill SE, Panavia, Prime & Bond with the samples actively irrigated and bonded using Clearfill SE, Panavia, Prime & Bond.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The current strategies for management of teeth requiring a post after endodontic therapy is based on the evolution of different adhesive materials and techniques combined with the ever-growing expertise and understanding of the clinician. Therefore, it is imperative to know these materials, techniques that various researchers have used and also, the difficulties faced over the years for the long-term survival of post retained root canal treated teeth.

Goldman et al in **1981** determined that the smeared layer is (1) caused by instrumentation, (2) calcific in nature, and (3) not removed by organic solvents or ampholytic soaps but is removed by a chelating agent (ethylene diaminetetracetic acid

[EDTA] 17%). They suggested the removal of smear layer by flushing the canal after instrumentation with 17% EDTA followed by 5.25% NaOCl. The chelating agent is followed by an organic solvent. They also reported 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.²²

Nakabayashi et al in **1982** described the hybrid layer as the interdiffusion zone of demineralized intertubular and peritubular dentin and polymerized resin and proposed this principle of micromechanical interlocking as a prime mechanism of bonding.²³

Madison and Zakariassen in **1984** found no statistically significant difference in linear apical dye leakage when gutta percha was removed by flame-heated endodontic pluggers or Peeso reamers when 5 mm of apical gutta percha remained.²⁴

In **1987**, **Watson and Boyde** for the first time described the use of fluorescent confocal microscopy for analysis of the interface of restorative materials and tooth structure. They advocated the use of fluorescent dyes, mixed into components of an adhesive system, to highlight the bonded interface.²⁵

Czonstkowsky et al in **1990** observed that the motor-driven instruments, such as Gates-Glidden or post drills, produced quantitatively more smear layer than hand files.²⁶

Van Meerbeek et al in **1992** conducted a research to evaluate the morphological aspects of the resin dentin interdiffusion zone (RDIZ) with different dentin adhesive

systems. He concluded that the application of these adhesive systems induced structural changes in the dentin surface morphology, creating a retentive interface, called the inter-diffusion zone, between the deep, untouched dentin layers and the composite filling material. This resin-dentin interdiffusion zone offers bonding sites for copolymerization with the resin composite and, concurrently, might have protective potential for pulp tissue as it blocks the normal passage of microorganisms and toxins.²⁷

Pashley et al in **1993** 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. They identified the surface porosities in dentin that permitted resin infiltration during dentinal bonding. 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.²⁰

Yoshiyama et al in **1996** reported that the self etch primers are more advantageous for bonding of fibre posts, since they contain a high concentration of acidic monomers that demineralize the substrate. Also, that the acid does not need to be removed with water, and the bond forms simultaneously to dentin.²⁸

Mjor A, Smith M, Ferrari M, Mannocci F in **2000** 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.²⁹

Mayhew et al in **2000** compared the effect of four irrigants on their bonding efficacy of resin cements to root canal dentin. They found that 50% citric acid ($C_6H_8O_7$) and 37% orthophosphoric acid (H_3PO_4) improved bond strength of resin cement to root canal dentin; whilst 5.25% NaOCl and 0.9% saline (NaCl) had no effect.

30

Ngoh et al in **2001** carried out a research aimed at analyzing the effects of eugenol on resin bond strength to root canal dentin. They compared the regional bond strength of a Metabond resin to root canal dentin with and without using a eugenol

containing sealer. The results of the study showed that the specimens treated with eugenol had lower bond strength when compared to those without eugenol. They also confirmed that eugenol interferes with the polymerization process and thus affects adhesive resin bonding agents and cements.³¹

Calt S and Serper A in 2002 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.³²

Ferrari et al in 2002 evaluated the effectiveness of a microbrush as a carrier of priming-adhesive solution in formation of resin tags, adhesive lateral branches, and RDIZ. Twenty endodontically treated teeth, already scheduled for extraction for endodontic or periodontal reasons, were selected for this study. The samples were randomly divided into 2 groups of 10 samples each. In group 1, One-Step (Bisco, Schaumburg, Ill) was applied with a brush whereas in group 2, One-Step was applied with a microbrush. A week after application, the root samples were extracted and processed for SEM observations. They observed that though both the adhesive systems

showed RDIZ, resin tag and adhesive lateral branch formation, the microbrush permitted a more uniform RDIZ and resin tag formation along the entire length of the canal than did the standard brush.³³

Serafino et al in **2004** 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. Forty teeth, divided into 4 groups, were instrumented, using Ni-Ti rotary files, irrigated with NaOCl or NaOCl + EDTA and obturated with cold lateral condensation (CLC) or warm vertical condensation (WVC) 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) not available for adhesive bonding and resin cementation of fiber posts. Hence its necessary to develop other procedures to achieve a dentinal canal wall better prepared for adhesive resin cementation in endodontically treated teeth.³⁴

Bitter et al in **2004** 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 cemento-enamel 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.¹⁹

Luz et al in **2005** investigated the dentin-resin interdiffusion zone using two different self-etching bonding systems, and compared them with a total-etch bonding system using a SEM. The descriptive analysis of the resin-dentin unions in the different self-etching resin bonding systems studied showed that all of them had formed an interdiffusion zone. However, each one of them presented its own characteristics as to thickness of the hybrid layer, formation of resin tags, and degree of its penetration, besides adhesive layer aspects. They proposed that these differences probably occurred because of the proper characteristics of each bonding system that ultimately determine

the degree of smear layer removal, underlying dentin demineralization, adhesive wettability, and diffusion through dentin. The application technique for each system could also have an influence.³⁵

Goracci et al in **2005** 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, Ivoclar-Vivadent) 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.³⁶

Garcia-Godoy et al. in **2005** 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.³⁷

Hayashi et al in **2006** studied the mode of fractures when extracted human premolar teeth were restored with fiber post, prefabricated metal post and cast metal post-core and subjected to oblique and vertical loads. They concluded that under the conditions of vertical and oblique loadings, the combination of a fiber post and

composite resin core with a full cast crown is most protective of the remaining tooth structure.³⁸

Menezes et al in **2008** evaluated the effect of composition of endodontic sealer and the time elapsed between root filling and post fixation on adhesion to root canal dentin.

Under the limitations of the study, the following conclusions were drawn:

- The calcium hydroxide-based endodontic sealer (Sealer 26) did not influence the pattern of bonding to root dentin irrespective of depth and time evaluated.
- 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.³⁹

Gu et al in **2009** carried out an invitro 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 scanning 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.⁴⁰

Malyk et al in **2010** 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.⁴¹

Vilanova et al.⁴² in **2012**, in contrary to **Garcia-Godoy et al.**, showed that 17% EDTA, when used as a final rinse for 5 min, produced high resin-dentin bond strength with the resin sealer.

They also rated different irrigants in achieving good bond strength with root dentin as:

1% NaOCl + 17% EDTA > 17% EDTA = 24% EDTA gel = 2% Chlorhexidine (CHX) gel > 1% NaOCl.

Marigo L et al in **2012** performed an in vitro morphometric evaluation of the resin root canal dentin interface of four total etch adhesive systems on 40 extracted human teeth. Within the limitations of the study, they concluded that all the adhesives, even if with different characteristics, produced good results in terms of HL thickness and RT density both at coronal and apical region. Out of the four etch and rinse adhesives, the three step adhesive engaged the canal dentin in a better way in terms of the thickest HL and a high density of RT, while the two step adhesives presented a shorter clinical protocol which could be useful during clinical practice.⁴³

Srirekha et al in **2013** 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 experimental groups. Group A was treated with 10 % citric acid followed by passive ultrasonic irrigation. Group B received a treatment with 17 % EDTA followed by PUI whereas Group C was conditioned with 36 % phosphoric without ultrasonic agitation. Group D (control) was treated with 3 % NaOCl and PUI. Samples were sectioned and evaluated for debris and smear layer removal under scanning electron microscope. Within the limitations of this study, the following conclusions were drawn;

- Coronal and middle third of the post space showed good smear layer and debris removal using citric acid and EDTA, along with ultrasonic agitation.

- In comparison with the coronal and middle third, apical third of the post space showed inadequate removal of debris and smear layer irrespective of the etching procedure or the irrigant (citric acid, EDTA) used.⁴⁴

Ekambaram et al in **2015** 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.
- The use of EDTA as a post space irrigant could enhance the bonding performance when self-etch adhesives are used to bond fibre post to internal root dentin.
- Literature shows mixed results on the bonding performances of various types of commercial adhesives to root dentin.

- Manufacturer's recommendation must be followed to achieve an optimum bond to root dentin with any type of dentin adhesives.⁴⁵

Giudice et al⁴⁶ in **2016** 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 every sample was assigned to one of three experimental groups and to one control group. In each group different irrigation protocols were performed as follows: EDTA (Group A), 37% orthophosphoric acid (Group B), and EDTA + 37% orthophosphoric acid with ultrasounds activation (Group C). In 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.

Within the limitations of the study, the following conclusions were drawn:

- 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.

Mohamed Awad in **2017** in his in vitro study assessed the RDI morphology of two ethanol based universal adhesives using SEM. He found that both the universal adhesives could penetrate the dentin forming well-defined resin tags and lateral branches. He concluded that both the tested adhesives were one-step self etch adhesives and water-ethanol combination in both the adhesives may have helped to dilute the viscous monomers and facilitate its infiltration into dentin.⁴⁷

Carvalho M et al in **2017** 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.⁴⁸

Ferreira JC et al in **2017** carried out a research that aimed to analyze the morphology of RDI yielded by two step etch and rinse adhesive with different solvents. A total of 32 dentin disks were prepared and randomly assigned to four groups: Group I- Adper Scotchbond-IXT (ethanol/water); Group II- XP-Bond (tertiary butanol); Group III- Prime and Bond NT (acetone); and Group IV- One Coat bond (5% water). SEM evaluation of the RDI revealed that adhesive systems with different solvents led to significant differences in the interface morphology. The adhesives containing tertiary butanol, seemed to originate a good quality hybrid layer, long and entangled tags and also appear to have a greater ability to originate microtags.⁴⁹

MATERIALS AND METHOD

MATERIALS & METHOD

One hundred and twenty freshly extracted human maxillary anteriors were selected for the study. 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 ethical committee was taken for the study.

SELECTION CRITERIA:

INCLUSION CRITERIA:

1. Sound maxillary anteriors with fully formed closed apices.
2. Teeth with single root and single canal.

EXCLUSION CRITERIA:

1. Teeth with caries, trauma, and/or fractures.
2. Teeth with developmental anomalies.
3. Teeth with internal or external resorption.
4. Teeth with severe curvatures.

ARMAMENTARIUM:

Instruments and Equipment:

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

- Digital Radiovisiography System (Kodak 5100 RVG, France)
- Gates Glidden drills (Mani, Japan) (PLATE–II)
- Standard 2% files # 10-80 (Mani, Japan) (PLATE–III)
- Reamers (Mani, Japan) (PLATE–III)
- Peeso drills (Mani, Japan) (PLATE–III)
- Mini Endo Bloc (DENTSPLY, Maillefer, Switzerland) (PLATE–II)
- Endodontic spreaders (Mani, Japan) (PLATE–III)
- Endodontic hand pluggers (Mani, Japan) (PLATE –III)
- Lentulospirals (Mani, Japan) (PLATE–III)
- Hot shot tip (Discus Dental, USA) (PLATE–IV)
- Endoactivator (Dentsply, Maillefer, Switzerland) (PLATE–IV)
- Endodontic Microbrush (PLATE–I)
- LED Light curing gun (3M ESPE, Germany) (PLATE–IV)
- L-moulds (PLATE–IV)
- Precision cutting saw (IsoMet 5000, Buehler, Germany) (PLATE–XII)
- Grinder polisher (Buehler, Germany) (PLATE–XII)
- Confocal laser scanning microscope (ZEISS with LSM Software ZEN 2007)
(PLATE–XII)

Materials:

- 5ml syringe with 24 gauge needle (Nirlife, India) (PLATE–V)
- Root canal irrigation solutions (PLATE–V)
 - Sodium hypochlorite (NaOCl) (Hyposept UPS Hygienes, India)
 - Normal saline (0.9 % w/v, Nirlife, India)

- Chelating agent for smear layer removal (PLATE–V)
 - 17% liquid Ethylene Diamine Tetra Acetic acid (EDTA) (DentWash, Prime Dental, India)
 - RC Help (Prime Dental Products, India)
- Paper points (DiaDent, Korea) (PLATE–V)
- Gutta Percha points (DiaDent, Korea) (PLATE–V)
- AH – Plus sealer (DENTSPLY, Maillefer, Switzerland) (PLATE –VI)
- Cavit (3M ESPE, Germany) (PLATE –VI)
- Fibre posts (Angelus, Brazil) (PLATE –VI)
- Rhodamine B dye (Loba Chemie, India) (PLATE –VI)
- Clearfill SE Bond (Kuraray, Japan) (PLATE –VII)
- Clearfill SA Luting (Kuraray, Japan) (PLATE –VII)
- Panavia F 2.0 (Kuraray, Japan) (PLATE –VII)
- Prime and Bond NT (DENTSPLY, Maillefer, Switzerland) (PLATE –VII)
- Calibra resin cement (DENTSPLY, Maillefer, Switzerland) (PLATE –VII)
- Conditioner 36 (DENTSPLY, Maillefer, Switzerland) (PLATE –VII)
- Auto polymerized clear Acrylic Resin (DPI – RR Cold Cure , Dental Products of India Ltd) (PLATE –VI)

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

SAMPLE PREPARATION

All teeth were decoronated at the cemento-enamel junction (CEJ) under copious water irrigation with a double sided diamond disc (DFS, Germany) to obtain a standardized length of $13\text{mm} \pm 1\text{mm}$. After decoronation, the coronal thirds of the canal were enlarged using Gates Glidden drills (Mani, Japan) using sizes 1- 4 in a descending order. The working lengths (WL) were visually established by subtracting 1 mm from the lengths of a size 15 K-file (Mani, Japan) when its tip appeared at the apical foramen. All roots were shaped uniformly at full working lengths to size 50 using reamers (Mani, Japan) with a reaming action and alternating Hedstro'm files (Mani, Japan) with a circumferential filing movement. This was followed by a stepback preparation to size 80. Throughout biomechanical preparation, after every change of file, irrigation was performed with 1 mL of 1% NaOCl solution using a 24 gauge needle.

The root canals were then dried with paper points and obturated by means of cold lateral condensation (CLC). Size 50 gutta percha points (Diadent, Korea) served as master cones and size 20 and 25 gutta-percha points were used as accessory points. AH plus (Dentsply, Maillefer, Switzerland) was used as a sealer in all the cases. Coronal surplus was removed with a Hot Tip (Discus Dental, USA) and the access cavities were temporarily filled with Cavit (3M ESPE, Germany) and stored for 24 h at 37 °C in 100% humidity.

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 9 mm for all the samples.

DISTRIBUTION OF STUDY GROUPS:

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

Groups	Sample Distribution	No. of Samples
Group I	Active Irrigation	60
Group II	Passive Irrigation	60

In **Active Irrigation** group, sonic irrigation i.e Endoactivator (Dentsply, Maillefer, Switzerland) with a blue colour coded tip 30/06 was used for activation of irrigants. The post space was irrigated with 1 mL of 5.25% NaOCl for 1 min followed by 1 mL of 17% EDTA for 1 min with sonic activation. An intermittent rinse was done with 0.9 % saline for 1 min.

In **Passive Irrigation** group, the post space was irrigated with 1 mL of 5.25% NaOCl for 1 min followed by 1 mL of 17% EDTA for 1 min without any activation. An intermittent rinse was done with 0.9 % saline for 1 min.

Upon completion of the respective irrigation protocol, the canals were rinsed with 0.9% saline and dried with multiple paper points. Fibre posts (Angelus, Brazil) were tried and the samples of each group were further divided into three sub groups according to the different adhesive luting system used.

GROUPS	SUB GROUPS	NO. OF SAMPLES
Group I Active Irrigation	I A. Clearfill SE Bond	20
	I B. Panavia 2.0	20
	I C. Prime And Bond NT	20
Group II Passive Irrigation	II A. Clearfill SE Bond	20
	II B. Panavia 2.0	20
	II C. Prime And Bond NT	20

The procedure for luting of posts with different adhesive resin systems was done according to manufacturer's instructions.

CLEARFILL SE GROUP

The root canal walls were dried with multiple paper points. Clearfill SE primer (Kuraray, Japan) was applied onto the root canal using a microbrush tip. Excess primer adhesive solution was removed with a paper point. Clearfil SE Bond (Kuraray, Japan) was labelled with 0.1% Rhodamine B dye (LobaChemie, India) and then applied using a microbrush and light cured for 10s. Clearfil luting cement (Kuraray, Japan) was applied into the root canal space with a lentulo drill (Mani,Japan) and also onto the post surface. Then, the posts were inserted into the root canal and light cured for 20 seconds using a LED light curing gun (3M ESPE, Germany) and excess cement was removed.

PANAVIA 2.0 GROUP

One drop of each Panavia ED Primer Liquid A & B (Kuraray, Japan) were mixed, labelled with 0.1% Rhodamine B dye (Loba Chemie, India) and applied with a microbrush for 15 s onto the root canal walls. The posts were then inserted using Panavia 2.0 cement, light cured for 20s and excess cement was removed.

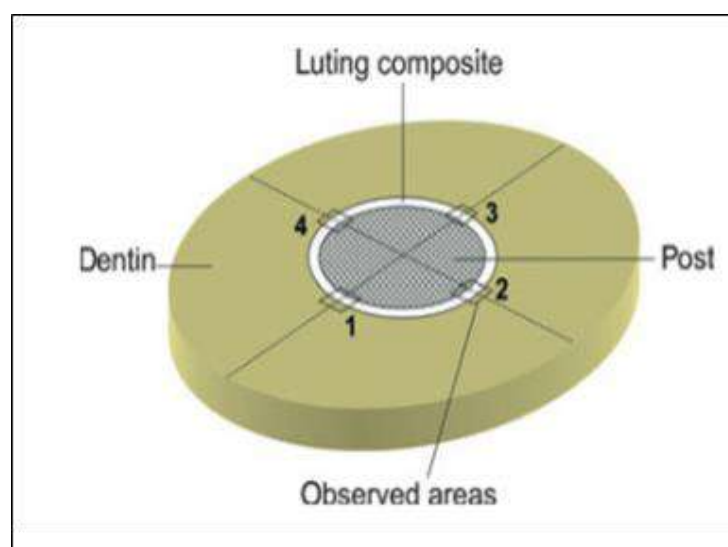
PRIME AND BOND NT GROUP

The root canal walls were conditioned with 36% phosphoric acid (Dentsply, Maillefer, Switzerland) for 15s, washed with water spray for 10s and gently air dried. Excess water was removed using paper points. Equal drops of Prime and Bond adhesive and self cure activator were mixed, labelled with 0.1% Rhodamine B dye (Loba Chemie, India) and applied onto the root canal walls with a microbrush for 20 s. 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 10s. 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 20s and excess cement was removed.

All samples were embedded in cold-cure acrylic resin using L-moulds of dimensions $1.5 \times 1.5 \text{ cm}^2$. Sections of the root were performed with a microtome precision saw (Isomet, Beuhler, Germany) 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 2 mm 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, Germany). The samples were kept humid during the whole study.

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 μm^2 , and the resolution was 512 x 512 pixel.



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

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 number of resin tags represented in the standardized images were counted.

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

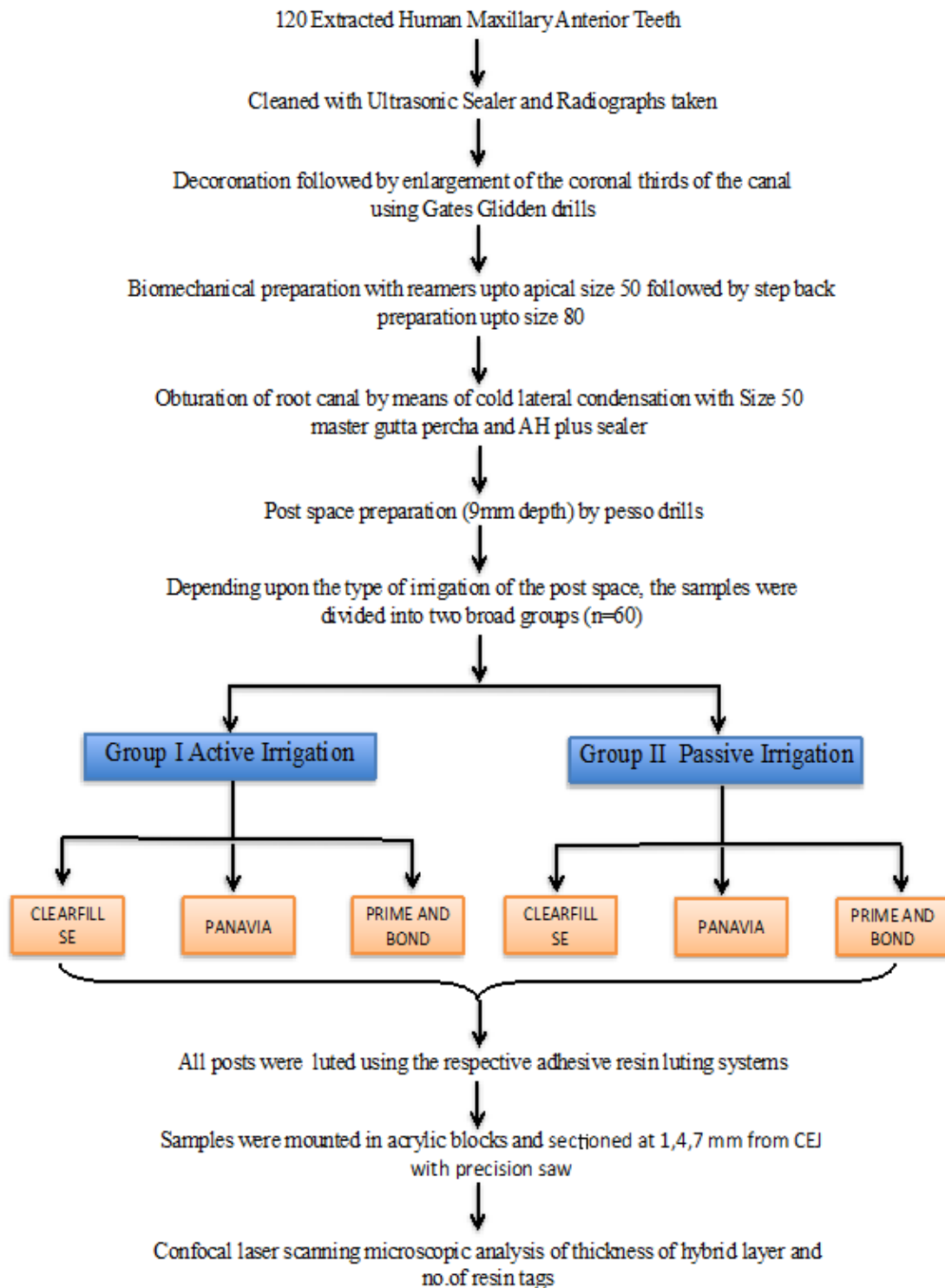


PLATE - I

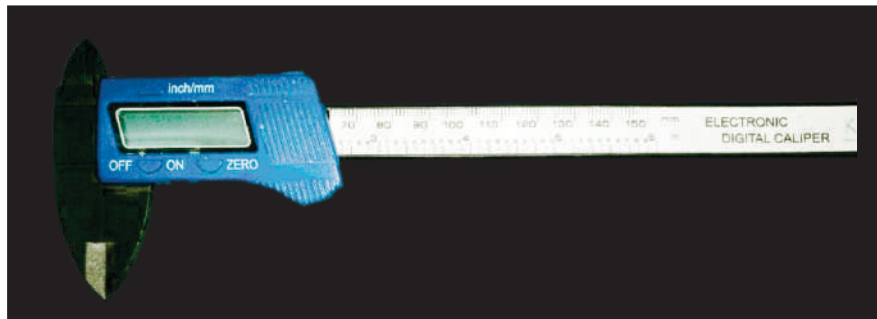
ARMAMENTARIUM



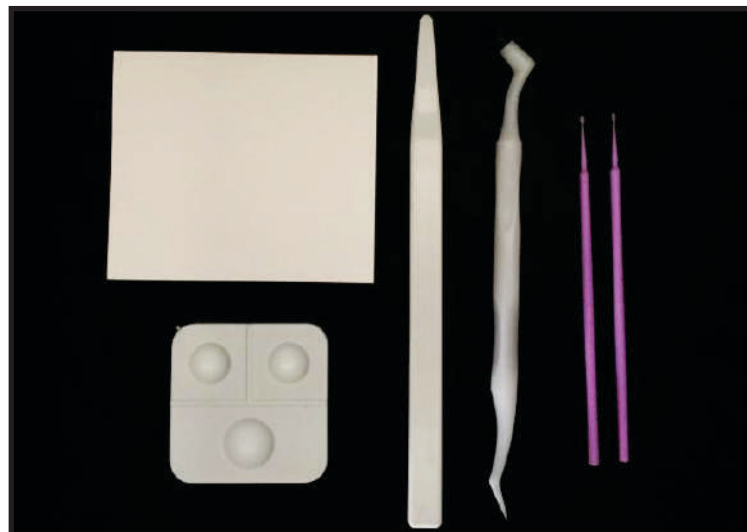
Hand Instruments
(GDC, India)



Cotton Holder & Waste Receiver
(GDC, India)



Digital Vernier Caliper
(Workzone tools, Germany)



Mixing pad, Spatula, Microbrushes, Dispenser

PLATE - II

ARMAMENTARIUM



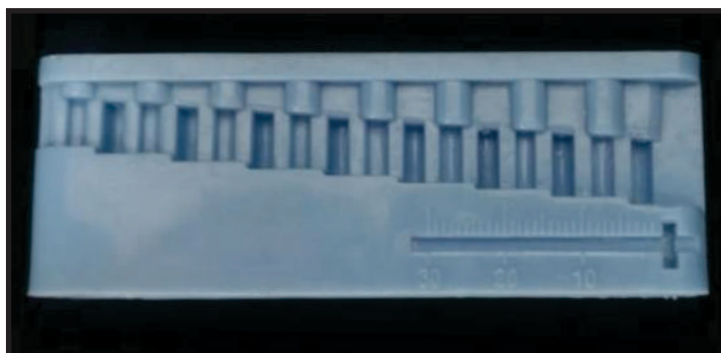
Straight handpiece , Double sided Diamond Disc
(NSK, Japan) (DSF, Germany)



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



Gates Glidden Drills
(Mani, Japan)



Endobloc
(DENTSPLY, Maillefer, Ballaigues, Switzerland)

PLATE - III

ARMAMENTARIUM



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



Reamers
(Mani, Japan)



Pluggers & Spreader
(Mani, Japan)



LentuloSpiral
(Mani, Japan)



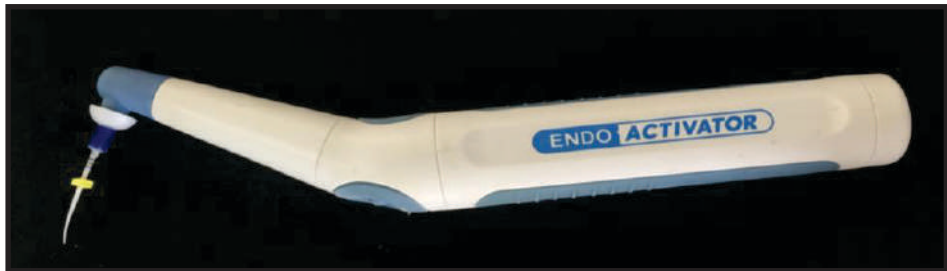
Peeso Drills
(Mani, Japan)

PLATE - IV

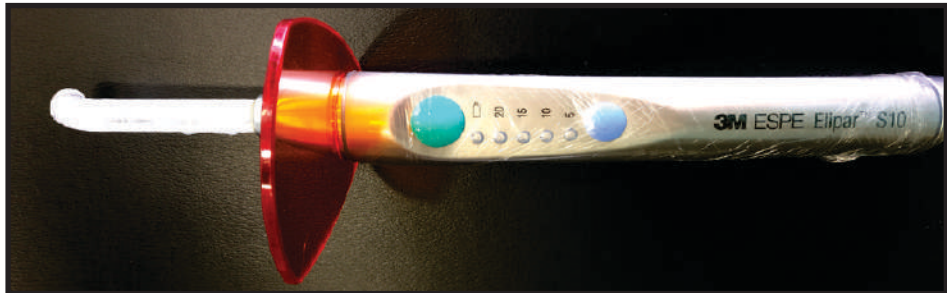
ARMAMENTARIUM



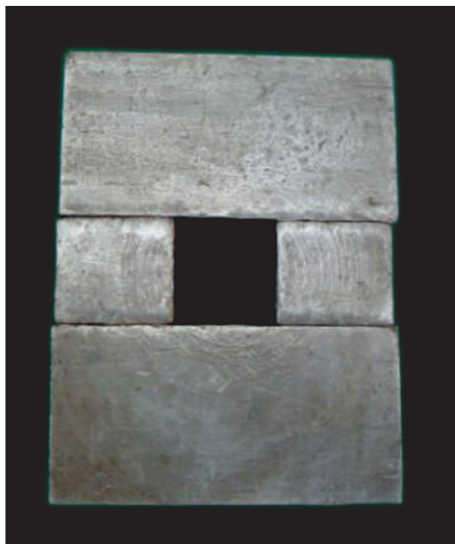
Hot Tip
(Discus Dental Inc. USA)



Endoactivator
(DENTSPLY, Maillefer, Ballaigues, Switzerland)



LED Light Curing Gun
(3M ESPE, Germany)



L-Mould



Grinder & Polisher
(Buehler, Germany)

PLATE - V

MATERIALS



Sodium Hypochlorite
(Hyosept UPS Hygiene, India)
Normal Saline
(Nirlife, India)



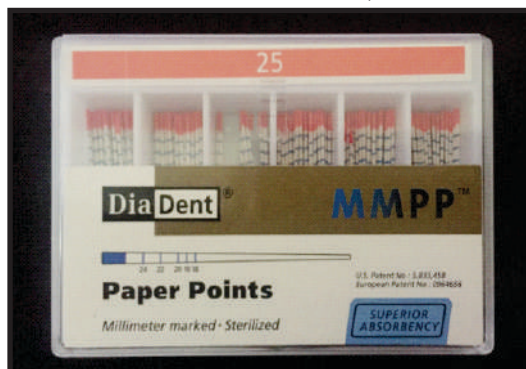
Dent Wash Liquid EDTA
(Prime dental, India)



Irrigation Syringe, Side Venting Needle
(Nirlife, India)



RC Help 17% EDTA Chelating gel
(Prime Dental Products, India)



Absorbant Points
(DiaDent, Korea)



Gutta Percha Points
(DiaDent, Korea)

PLATE - VI

MATERIALS



AH Plus Sealer
(Dentsply, Maillefer, Ballaigues, Switzerland)



Cavit G
(3M ESPE, Germany)



Rhodamine B Dye
(Loba chemie, India)



Fibre post
(Angelus, Brazil)



Autopolymerized Acrylic Resin
(DPI-RR Cold Cure)

PLATE - VII

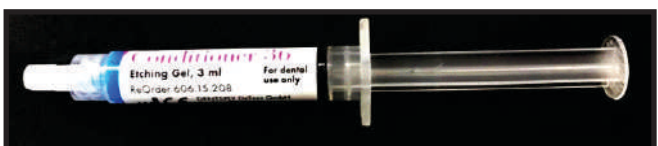
MATERIALS



Group A - Clearfill
(Kuraray Noritake, Japan)



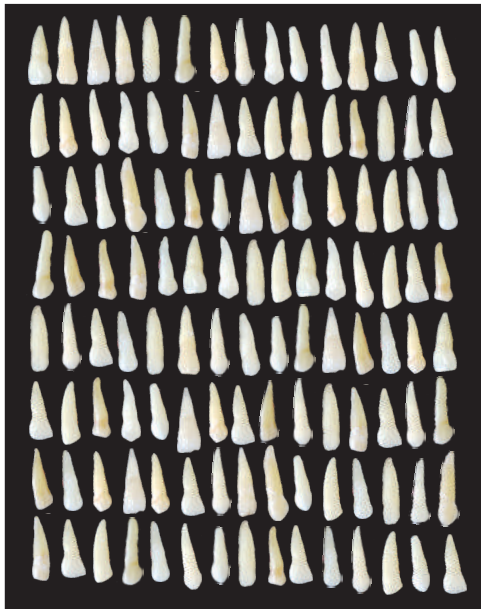
Group B - Panavia
(Kuraray Noritake, Japan)



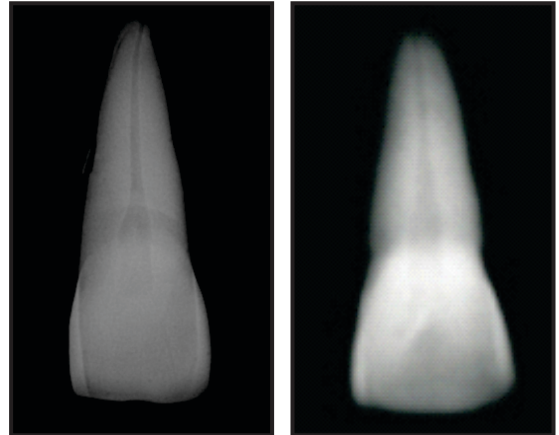
Group C - Prime & Bond NT, Calibra Resin Cement
(Dentsply, Maillefer, Ballaigues, Switzerland)

PLATE - VIII

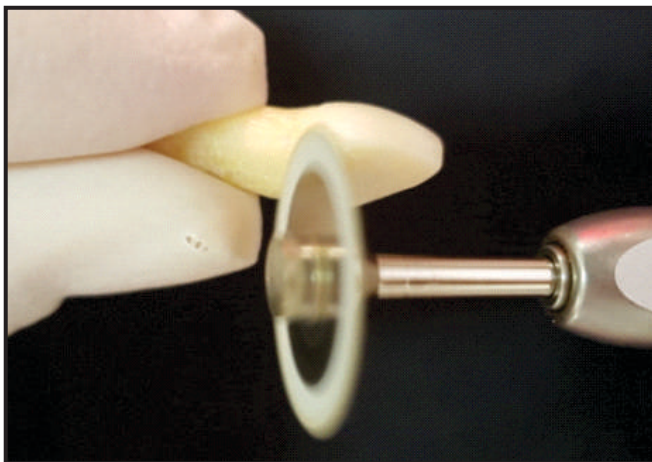
METHODOLOGY



Sample Size (N=120)



Pre Operative Radiographs



Decoronation of Samples



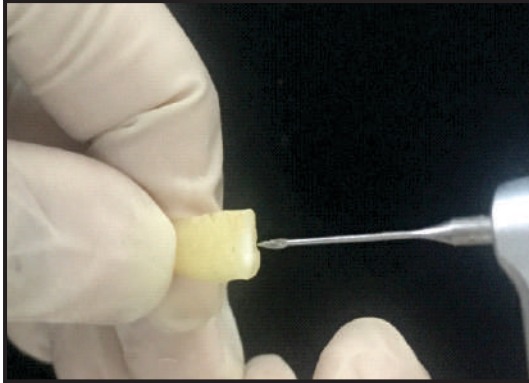
Decoronated samples (n=20)



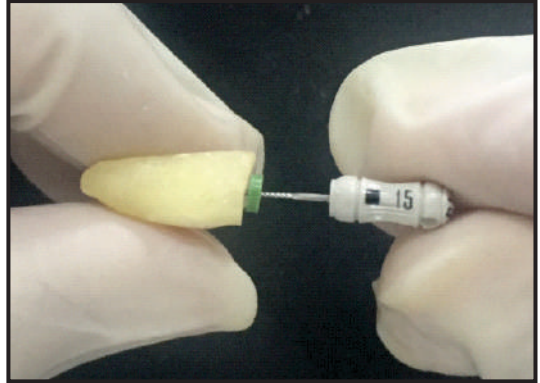
Length Measured with Digital Vernier Caliper

PLATE -IX

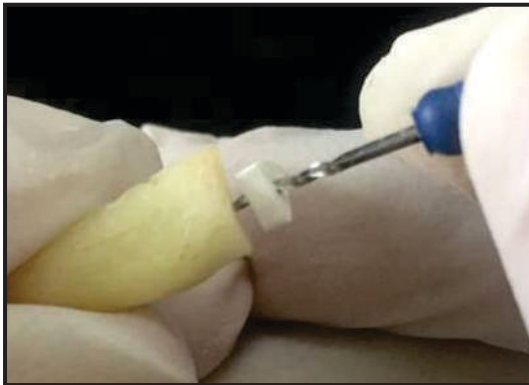
METHODOLOGY



**Coronal enlargement by
Gates Glidden Drill**



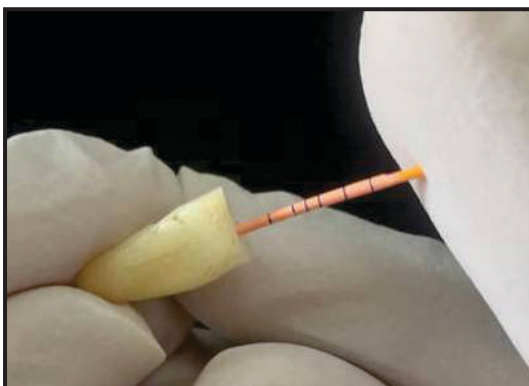
Working length Determination



Biomechanical Preparation



Irrigation of root canal



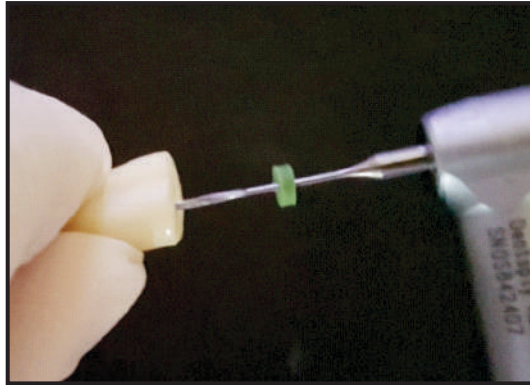
Master cone selection



Obtured Root Canal

PLATE - X

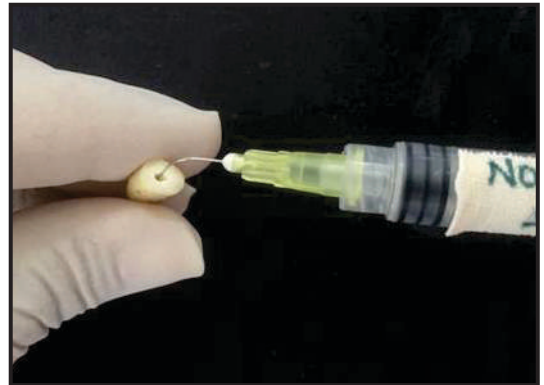
METHODOLOGY



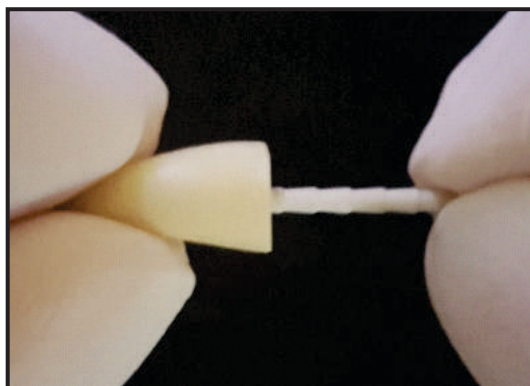
Post Space Preparation



**Group I:
Post Space Active Irrigation**



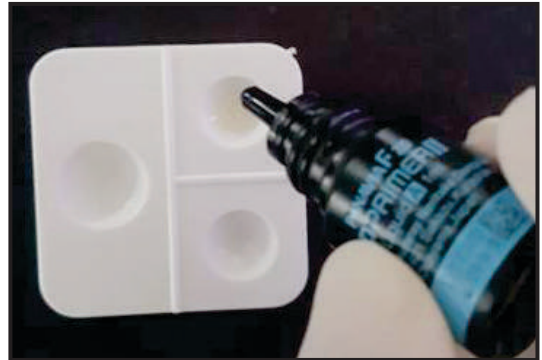
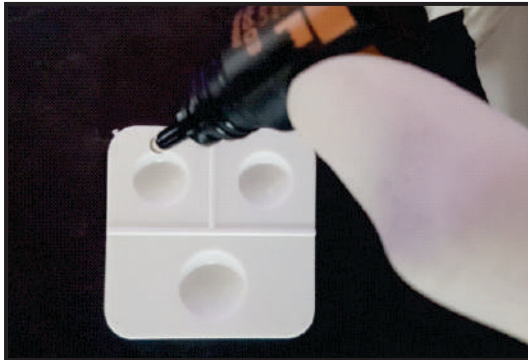
**Group II:
Post Space Passive Irrigation**



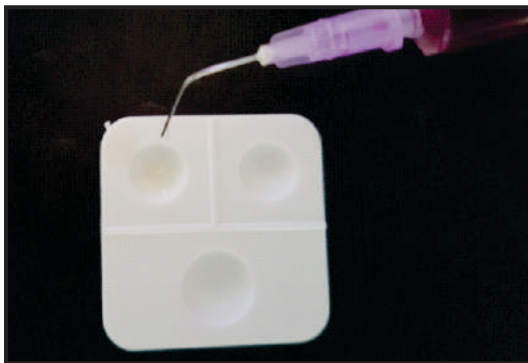
Trial of Fibre Post

PLATE - XI

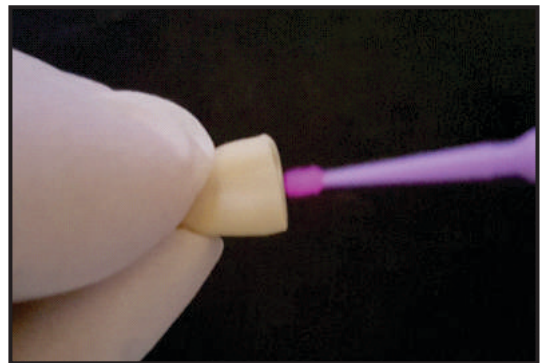
LUTING OF FIBRE POST



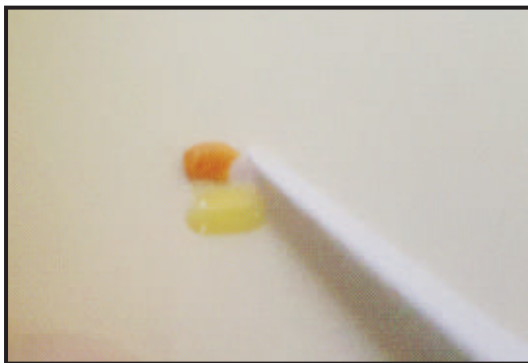
Dispensing & Mixing of Primer I & II



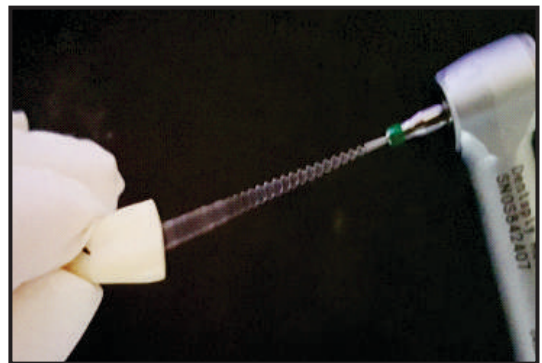
Labelling with Rhodamine B dye



Application of Primer with Microbrush



Manipulation of Luting Cement



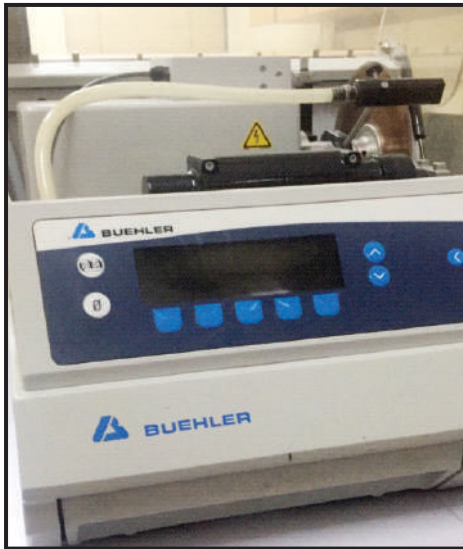
Application of Luting Cement with Lentulo Drill



Seating of Fibre post & Curing

PLATE - XII

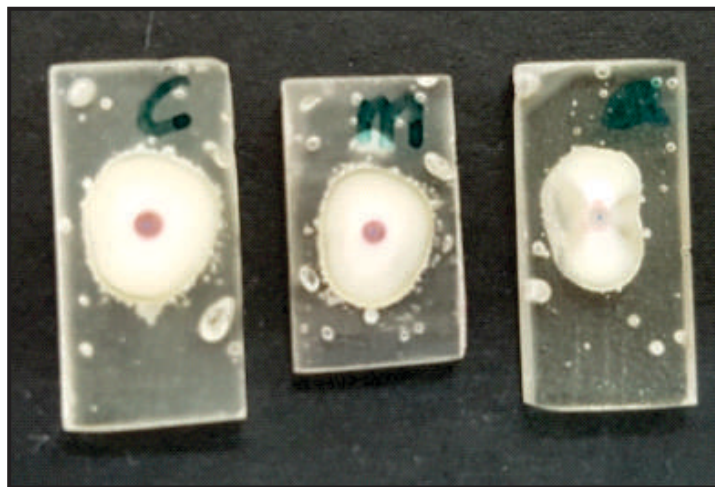
METHODOLOGY



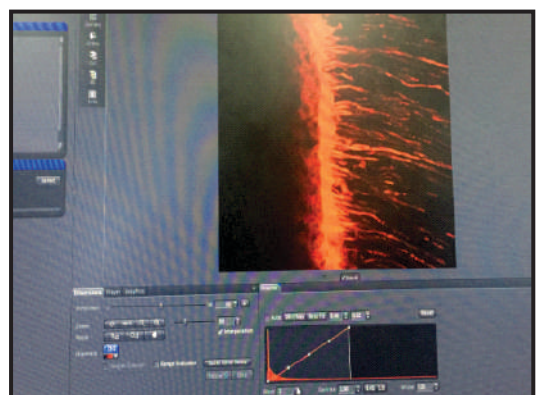
**Sectioning of samples with Isomet Precision Saw
(Buehler, Germany)**



**Polishing of samples on Grinder & Polisher
(Buehler, Germany)**



Sectioned Samples



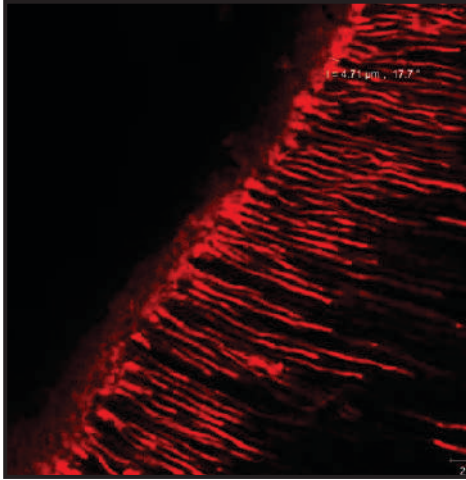
**Confocal Laser Scanning Microscope
(LSM 510, GIESS with LSM Software ZEN 2007)**

PLATE - XIII

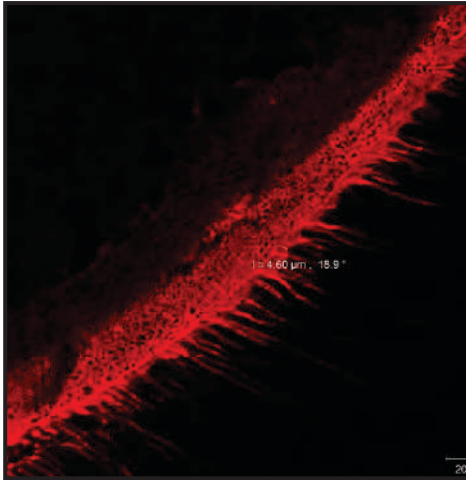
CONFOCAL LASER MICROSCOPIC IMAGES

GROUP A

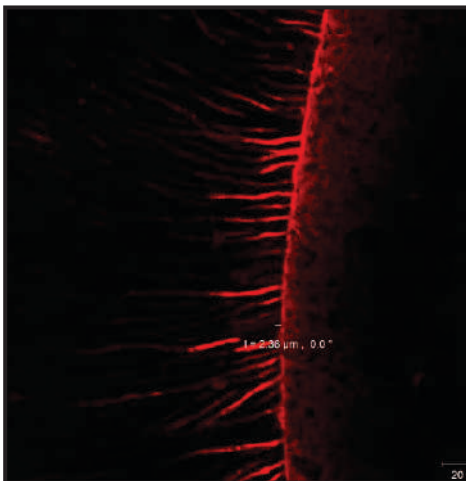
**Group I ACTIVE IRRIGATION
CORONAL**



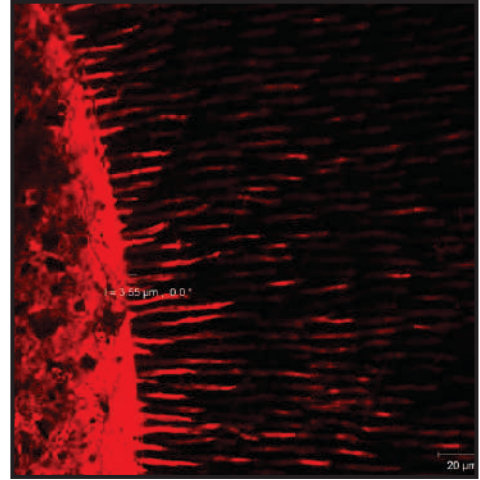
MIDDLE



APICAL



**Group II PASSIVE IRRIGATION
CORONAL**



MIDDLE



APICAL

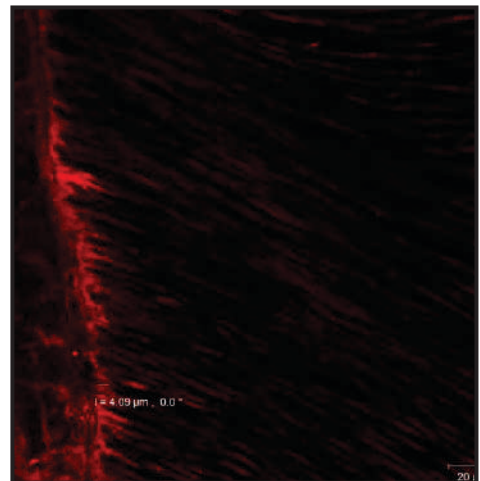
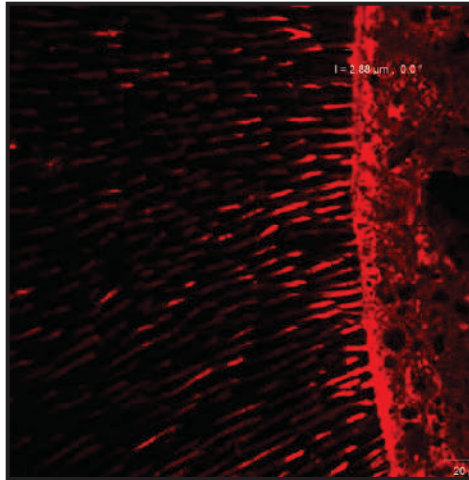


PLATE - XIV

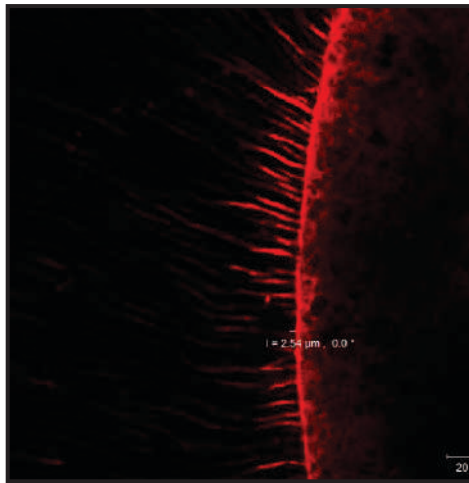
CONFOCAL LASER MICROSCOPIC IMAGES

GROUP B

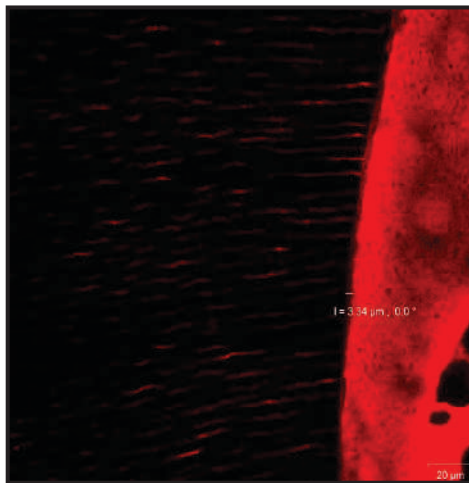
**Group I ACTIVE IRRIGATION
CORONAL**



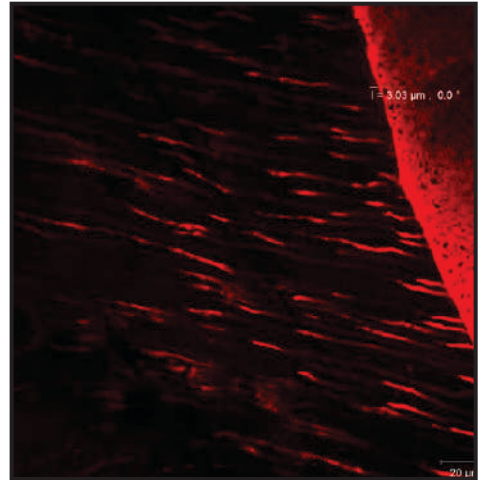
MIDDLE



APICAL



**Group II PASSIVE IRRIGATION
CORONAL**



MIDDLE



APICAL

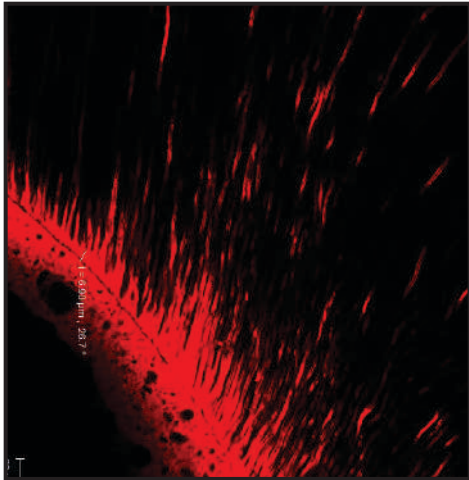


PLATE - XV

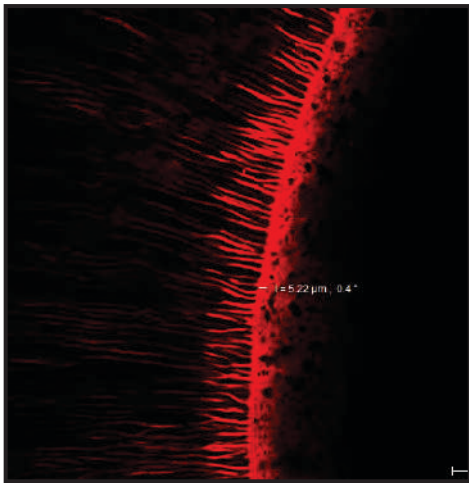
CONFOCAL LASER MICROSCOPIC IMAGES

GROUP C

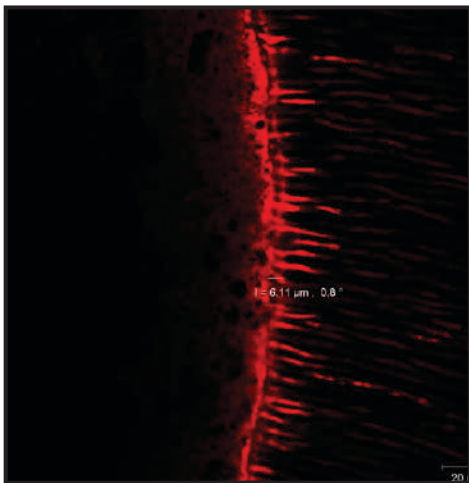
**Group I ACTIVE IRRIGATION
CORONAL**



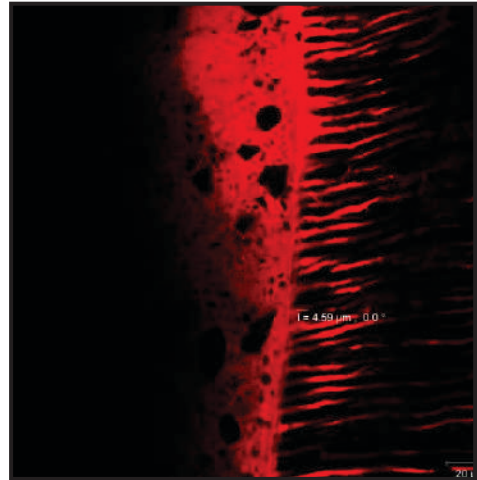
MIDDLE



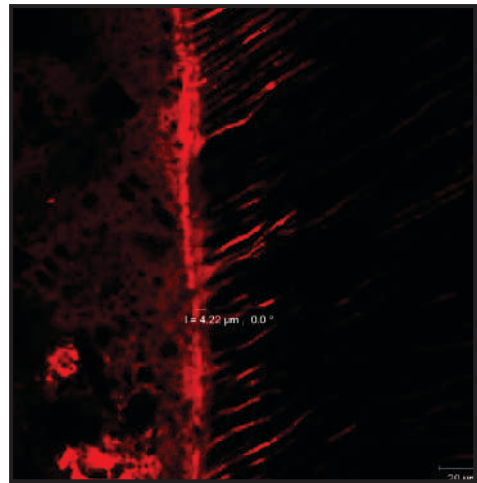
APICAL



**Group II PASSIVE IRRIGATION
CORONAL**



MIDDLE



APICAL



RESULTS

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 systems and the effect of active and passive irrigation on them by confocal laser scanning microscope.

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

GROUP I - Active Irrigation

GROUP II - Passive Irrigation

Upon completion of post space irrigation, fibre posts were tried and the samples of each group were further sub divided into three sub groups according to the different adhesive luting systems used.

Thus the final groups and sub groups were as follows :

GROUPS	SUB GROUPS
GROUP I ACTIVE IRRIGATION	I A. CLEARFILL SE BOND I B. PANA VIA 2.0 I C. PRIME AND BOND NT
GROUP II PASSIVE IRRIGATION	II A. CLEARFILL SE BOND II B. PANA VIA 2.0 II C. PRIME AND BOND NT

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) and number of resin tags.

STATISTICAL ANALYSIS:

Licensed version of SPSS version 20.0 (IBM Corp) was used for statistical analysis. The data on two factors i.e. thickness of hybrid layer and number of resin tags were obtained for tooth exposed to two different irrigation types i.e. Active and Passive Irrigation (Group I & II) and three different adhesive luting systems i.e. Clearfill SE (Group A), Panavia 2.0 (Group B), Prime and Bond NT (Group C). Descriptive statistics like mean and standard deviation were obtained for each section of tooth according to adhesive luting systems and irrigation type.

Two-way analysis of variance was used to determine the effect of irrigation types and adhesive luting systems on the dependent variables i.e. thickness of hybrid layer and number of resin tags for each section independently. The interaction effect between the two fixed effects was observed and accordingly the main or simple main effect of factors was reported. Materials showing statistical significant effect on the dependent variables (hybrid layer and resin tags) were further evaluated using Tukey's post-hoc test. The statistical significance was tested at 5% level.

The details of the methods used are as below:

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

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

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

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

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

where x_i = observation on each object

n = number of objects

C) **Two-way ANOVA**

Let there be two factors A and B at a and b levels respectively. Let x denote the variable under study, such that x_{ijk} ($i=1, 2, \dots, a$; $j=1, 2, \dots, b$, $k=1, 2, \dots, r$) represent the k^{th} observation from i^{th} level of factor A and j^{th} level of factor B.

The two-way ANOVA will test for the main effects of factor A or B, namely,

$H_0: \mu_{1.} = \mu_{2.} \dots = \mu_{a.}$ for factor A and

$H_1: \mu_{1.} = \mu_{2.} \dots = \mu_{b.}$ for factor B

Finally, each observation x_{ijk} can be expressed as $x_{ijk} = \mu + a_i + b_j + c_{ij} + e_{ijk}$

where e_{ijk} denotes the error (unexplained component).

The two factors A and B are said to fixed factors and the model is the fixed effects model. For such model with equal number of observations per cell, the total sum of squares is given by the expression:

$$SS(\text{total}) = SS(A) + SS(B) + SS(AB) + SSE$$

where AB is the interaction effect due to factors A and B.

Various formulae for estimating these sums of squares are as below:

The total sum of squares is given by

$$SS(\text{total}) = \sum_i \sum_j \sum_k (x_{ijk} - \bar{x} \dots)^2 \text{ with } n-1 \text{ degrees of freedom.}$$

Sum of squares due to factor A is given by

$$SSA = rb \sum_i (\bar{x}_{i.} - \bar{x} \dots)^2 \text{ with } a-1 \text{ degrees of freedom.}$$

Similarly, sum of squares due to factor B is given by

$$SSB = ra \sum_j (\bar{x}_{.j} - \bar{x} \dots)^2 \text{ with } b-1 \text{ degrees of freedom.}$$

Sum of squares due to interaction is given by

$$SS(AB) = r \sum_i \sum_j (x_{ij.} - \bar{x}_{i.} - \bar{x}_{.j} + \bar{x} \dots)^2 \text{ with } (a-1)(b-1) \text{ degrees of freedom}$$

And sum of squares due errors is given by

$$SSE = \sum_i \sum_j \sum_k (x_{ijk} - \bar{x}_{ij.})^2 \text{ with } (n-ab) \text{ degrees of freedom}$$

Accordingly, the mean sum of squares are:

$$MST = SS(\text{total}) / (n-1); \quad MSA = SSA / (a-1); \quad MSB = SSB / (b-1);$$

$$MS(AB) = SS(AB) / (a-1)(b-1) \quad MSE = SSE / (n-ab)$$

The F-statistic for each factor could be obtained as:

$$F_{(a-1, (n-ab))} = MSA / MSE \text{ for factor A,}$$

$$F_{(b-1, (n-ab))} = MSB / MSE \text{ for factor B and interaction effect}$$

$$F_{((a-1)(b-1), (n-ab))} = MS(AB) / MSE$$

Thus, the decision of accepting or rejecting the null hypothesis can be taken from F-statistic.

D) Tukey's post-hoc test

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

$$\text{It is given by: } HSD = q \sqrt{\frac{MS_{within}}{n}}$$

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.

OVERALL RESULTS:

The mean values and standard deviation of thickness of hybrid layer and no. of resin tags for two main groups; Active Irrigation (Group I) and Passive Irrigation (Group II); for three different adhesive luting systems namely Clearfill SE (Group A), Panavia 2.0 (Group B), Prime and Bond NT (Group C) at Coronal, Middle and Apical sections have been described in Table 1 and Table 5.

The maximum thickness of hybrid layer (6.40 ± 0.75) was observed in Group I C at coronal level whereas the minimum thickness of hybrid layer (1.70 ± 0.66) was observed in Group II B. The maximum resin tags (21.30 ± 2.60) were observed in Group I C at coronal level whereas the minimum resin tags (5.20 ± 1.06) were observed in Group II B at apical level.

Higher values for thickness of hybrid layer and no. of resin tags were obtained for Active Irrigation group as compared to those in Passive Irrigation group. Higher values for thickness of hybrid layer and no. of resin tags were obtained for groups in which Prime and Bond NT was used as compared to those where fibre posts were luted with Clearfill SE and Panavia 2.0, irrespective of the irrigation protocol used. Higher values for thickness of hybrid layer and no. of resin tags were also obtained at coronal section as compared to middle and apical sections irrespective of whether active or passive irrigation was used for the three different adhesive luting systems. (Table 1 and 5, Figures 1-3 and Figures 8-10)

ANALYSIS FOR THICKNESS OF HYBRID LAYER

In order to determine the effect of post space irrigation (PSI) and different adhesive luting systems on thickness of hybrid layer, two-way analysis of variance was performed with groups and subgroups as fixed effects. This analysis was performed at coronal, middle and apical sections, independently. (Table 2, Table 3, Table 4)

At coronal section, the interaction between groups and subgroups was highly significant (p- value < 0.0001). (Table 2) Reporting these interactions directly, could be misleading. Therefore, alternatively, the main effect of groups corresponding to each type of adhesive luting system and subgroups were obtained. (Table 2a, Table 2b)

The simple main effects were statistically highly significant for each adhesive luting system as indicated by p-values < 0.0001. In other words, there was a statistically significant difference in the mean thickness of hybrid layer in active and passive irrigation types irrespective of the type of adhesive luting system used. The mean level for active irrigation was significantly higher than passive irrigation for all the three adhesive luting system. (Figure 4)

Table 2b shows that there is a statistically significant difference in the mean thickness of hybrid layer of the three different adhesive luting systems irrespective of the type of irrigation. The mean for Group C was significantly higher than Group A and B, and this finding was consistent for both the types of irrigation. (Figure 5).

At Middle section, the interaction between the groups and subgroups was statistically insignificant (p -value = 0.266) and hence, the main effects of groups and subgroups were reported individually. (Table 3, 3a, 3b)

The mean thickness of hybrid layer for Clearfill SE was 3.25 μm , for Panavia was 3.05 μm and for Prime and Bond NT was 5.57 μm . The difference in the means was highly significant with p -value < 0.0001 (Table 3). Hence, pair wise comparison was performed using Tukey's post-hoc test. (Table 3c)

The mean difference between Group A and Group B was statistically insignificant (p -value = 0.505). However, the differences between Group A and Group C, as well as between Group B and Group C were statistically highly significant (p -values < 0.0001). (Figure 6)

At Apical section, the interaction between the groups and subgroups was statistically insignificant (p -value = 0.622) and hence, the main effects of groups and subgroups were reported individually. (Table 4, 4a, 4b)

The mean thickness of hybrid layer was 4.21 μm for active and 2.63 μm for passive irrigation types considering all adhesive luting systems for Apical sections. The difference between the two means was highly significantly different. (p -value < 0.0001) (Table 4a)

The difference in the means of thickness of hybrid layer for the three adhesives considering irrigation type for Apical sections was highly significant with p -value < 0.0001 (Table 4). Hence, pair wise comparison was performed using Tukey's post-hoc test which showed a statistically insignificant result between Group A and Group B (p -

value = 0.362) and a statistically highly significant result between Group A and Group C and Group B and Group C (p-value < 0.0001) (Figure 7)

ANALYSIS FOR NUMBER OF RESIN TAGS

In order to determine the effect of post space irrigation (PSI) and different adhesive luting systems on no. of resin tags, two-way analysis of variance was performed with groups and subgroups as fixed effects. This analysis was performed at coronal, middle and apical sections, independently. (Table 6, Table 7, Table 8)

At Coronal section, the interaction between the groups and subgroups was statistically insignificant (p-value = 0.057) and hence, the main effects of groups and subgroups were reported individually (Table 6, 6a, 6b). Mean value for number of resin tags for Active irrigation was 16.8 and Passive irrigation was 12.55 considering all subgroups. The difference between the two means was highly significantly different as indicated by p-value < 0.0001 (Table 6).

The mean number of resin tags for Group A was 14.45, for Group B was 10.75 and for Group C was 18.82. The difference in the means was highly significant with p-value < 0.0001 (Table 6). Hence, pair wise comparison was performed using Tukey's post-hoc test. (Table 6c)

The mean difference between Group A, Group B and Group C was statistically significant (p-value < 0.001). (Figure 11)

At Middle section, the interaction between the groups and subgroups was statistically insignificant (p-value = 0.069) and hence, the main effects of groups and subgroups were reported individually. (Table 7, 7a, 7b)

The mean of number of resin tags was 13.46 for active and 9.75 for passive irrigation types considering all adhesive luting systems for Middle sections. The difference between the two means was highly significantly different. (p-value < 0.0001) (Table 7a)

The difference in the means of number of resin tags for the three adhesives considering irrigation type for Middle sections was highly significant with p-value < 0.0001 (Table 4). Hence, pair wise comparison was performed using Tukey's post-hoc test which showed a statistically significant result between Group A, Group B and Group C (p-value <0.0001) (Figure 12)

At Apical section, the interaction between groups and subgroups was highly significant (p- value < 0.0001). (Table 8) Reporting these interactions directly, could be misleading. Therefore, alternatively, the main effect of groups corresponding to each type of adhesive luting system and subgroups were obtained. (Table 8a, Table 8b)

The simple main effects were statistically highly significant for each adhesive luting system as indicated by p-values < 0.0001. In other words, there was a statistically significant difference in the number of resin tags in active and passive irrigation types irrespective of the type of adhesive luting system used. The mean level for active irrigation was significantly higher than passive irrigation for all the three adhesive luting system. (Figure 13)

Table 8b shows that there is a statistically significant difference in the mean of number of resin tags of the three different adhesive luting systems irrespective of the type of irrigation. The mean for Group C was significantly higher than Group A and B, and this finding was consistent for both the types of irrigation.

DISCUSSION

DISCUSSION

An endodontically treated tooth can only be salvaged as a functional unit within the oral cavity after successful root canal therapy if an adequate post endodontic restoration is placed over the tooth. In fact, few researches⁵²⁻⁵⁵ have shown that the post endodontic restoration has a greater impact on the outcome of endodontic therapy than the endodontic filling itself.

Modern restoration of devitalised teeth with a significant loss of coronal tooth structure often requires a post and core system.^{56,57} Until 2000, cast post-and-core was the most commonly used post type. Unfortunately, several disadvantages are associated

with conventional cast post-and-cores, such as loss of post retention, root fractures, and risk of corrosion.

Duret et al in **1990** introduced fibre posts to dentistry and provided an alternative to cast or prefabricated metallic posts for the restoration of endodontically treated teeth.¹⁹ Fibre posts have two important characteristics: (1) the modulus of elasticity of the fibre posts is similar to that of dentin⁶ and (2) these posts are cemented with an **adhesive technique** to avoid frictional retention between the post and root canal walls.⁵⁸

The bonding principle of dental adhesives to root canal dentin is based on the formation of a hybrid layer (**Nakabayashi et al. 1991**¹²) as well as the penetration of adhesive into the dentinal tubules i.e resin tags (**Titley et al. 1995**¹³, **Ferrari & Davidson 1996**¹⁴). The interdiffusion zone of demineralized intertubular and peritubular dentine and polymerized resin was first described by **Nakabayashi** and is essential for an ideal bond to dentin (**Nakabayashi et al. 1992**⁵⁹). The formation of the hybrid layer and resin tags is dependent on the penetration qualities and surface behaviour of various adhesive systems as well as on the condition and permeability of the dentinal surface (**Walshaw & McComb 1994**⁶⁰).

Though, adaptation of adhesive systems for fibre post bonding in root canal is an attractive clinical concept, its implementation is controversial for several reasons⁷⁻¹¹: influence of the endodontic procedure, polymerization shrinkage (**Feilzer et al. 1993, Carvalho et al. 1996**), unfavourable cavity configuration factor (C- Factor)

(**Tay et al. 2005**), poor control of moisture (**Bouillaguet et al. 2003**) or polymerization difficulties in the apical regions (**Roberts et al. 2004**).

Previous morphologic researches have also shown that bonding to root canals may be influenced by the variability of intraradicular dentin (**Ferrari et al 2000**¹⁶; **Mannocci et al 2001**⁶¹; **Serafino et al 2004**³⁴), endodontic procedures prior to post cementation such as post space irrigation (**Ngoh et al 2001**³¹), and the different dentin adhesives used.

Thus, achievement of reliable bonding and effective adhesion inside the root canal is still an issue of interest. No previous research has conclusively remarked on the effect of post space irrigation, variability of root canal dentin and different adhesives on hybrid layer and resin tag formation. Hence, the following study was carried out to evaluate the hybrid layer and resin tags at resin dentin interface in three different adhesive resin luting systems (total etch v/s self etch) and the effect of active and passive irrigation on them using Confocal Laser Scanning Microscopy (CLSM).

One hundred and twenty freshly extracted human maxillary anteriors were selected for the study. The teeth were collected and stored in phosphate-buffered saline for not more than 12 weeks as suggested by **Jameson MW et al (1994)**⁵¹. He had observed that storage media and time of the specimen storage affect the tooth after extraction due to water loss with dehydration of dentin. Phosphate-buffered saline shows the best compatibility in maintaining the hydration of the extracted teeth.

For sample size estimation, a study by **Bitter et al in 2004**¹⁹ was referred. The maximum thickness of hybrid layer as seen in Clearfill Universal i.e 5.45 um while

least was seen in Panavia i.e 0.84 μm . Assuming that similar differences could be obtained in the present study, the estimated sample size that could provide 90% power was 20 samples per group. Therefore, the total sample size for the current research was kept 120.

Maxillary anteriors are more prone to traumatic dental injuries (TDIs) owing to their location in the dental arch. **Goyal et al**⁶² in **2017** reported that 72.48% of all TDIs affected the maxillary arch. Crown fractures are the most common of all dental injuries. Complicated coronal fractures of anterior teeth often require esthetic rehabilitation that mandates the placement of a fibre post. Hence in the current study, maxillary anteriors were selected as samples.

The anatomic crowns of all the teeth were sectioned perpendicular to the long axis of the tooth with a water cooled diamond disc 2 mm above the cemento-enamel junction (CEJ), so that the coronal surface was perpendicular to the long axis of the root, and the remaining root length was 13 ± 1 mm and the cut coronal surface was ground flat using an abrasive paper. This led to standardization and uniformity in samples.¹⁹

One of the methods of measuring the working length on extracted teeth is to insert an endodontic file into the root canal until the tip of the file was just visible at the apical foramen. The stopper was adjusted to the reference point and the file was withdrawn. The canal length was determined and the working length was established by deducting 1 mm from this length; these readings were registered as actual working length (AWL). This method as described by **Shanmugaraj M et al** was employed in the study.⁶³

Success of any endodontic treatment depends on strict adherence to ‘endodontic triad’. Preparation of root canal system is recognized as being one of the most important stages in the root canal treatment. Decision on the technique of biomechanical preparation depends upon the geometry of root canal. Maxillary anteriors present with variations in canal cross section shapes from cervical to apical region. **Rodig et al.** determined that nickel–titanium rotary instruments did not allow controlled preparation of the buccal and lingual extensions of ovoid canals. Also, one of the limitations of rotary file system is that the largest size available may not conform to the root canal anatomy of maxillary anteriors. Hence in the present study, hybrid technique of root canal preparation (crown down by gates glidden drills and step back by 2% hand files) was done so as to achieve a gradual and well tapered canal preparation.⁶⁴

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 et al.**⁶⁵ and **Teixeira et al.**⁶⁶ showed that the root canal sealers, based on their chemical composition, have an effect on bonding of fibre posts to root canal dentin. **Schwartz et al.**⁶⁷ reported that eugenol, which remained on the surface of root canal dentin, affected polymerization of resin adhesives and interfered with resin penetration into the dentin matrix. Therefore, a resin based sealer AH plus was used in the study.

Preservation of apical seal is an important factor in determining the prognosis of teeth that are to be restored with post and core. Failure to maintain and preserve the apical seal may result in bacterial invasion leading to reinfection. **Wu et al**⁶⁸ suggested

that the percentage of sealer-coated canal perimeter was influenced by different condensation procedures and it was higher after lateral condensation than after vertical condensation. In the present study, thus obturation of the root canal was done by cold lateral condensation (CLC) using a 50/0.02 master cone and accessory cones.

After obturation, the post space preparation was done with peeso drills and a post space depth of 9 mm was kept for all the samples for standardization. In this study, peeso drills were used as their rotary action creates parallel walls, which provide optimal retention for the post.

Czonszkowsky et al.²⁶ observed that the motor-driven instruments, such as peeso post drills, produced quantitatively more smear layer than hand files. **Serafino et al.**³⁴ stated that post space preparation led to a thick smear layer that routinely contained remnants of dentin, sealer and gutta-percha. This thick smear layer could affect the bonding of fibre post to root canal dentin. **Coniglio et al.**⁶⁹ opined that post space preparation produced a thick smear layer and that just rinsing the post space with water or saline alone before bonding might not be sufficient.

To achieve an optimal rehabilitation, it is thus necessary to achieve a clean, smear layer free post space area and, secondly, to make sure that the irrigants are compatible with the subsequent cementation method of the post to be used. In the present study, after post space preparation, the samples were divided into two broad groups which received the same irrigants but with either activation or without it. (Group I- Active Irrigation and Group II-Passive Irrigation)

Previous researches^{34,47} on endodontic treatment have shown that 17% EDTA and 5.25% NaOCl when used alternately could remove the smear layer effectively. However, such usage of irrigation or even EDTA alone for 5 minutes would lead to severe erosion on the radicular dentin surface. **Saito et al** found that shortened irrigation time with EDTA less than 1 minute could significantly decrease smear layer removal. Thus, in our study, 1 ml of each 17% EDTA and 5.25%NaOCl were used for post space irrigation (PSI) alternately with a 1-minute irrigating time as also suggested by **Calt and Serper**.³²

The effect of residual irrigants in the root canal should also be taken into account. 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 rinsed with 0.9% saline.^{70,71,72}

The introduction of sonic and ultrasonic technology in endodontics has led to the proposal of new irrigation protocols for cleaning of post space. **Zhang et al**⁷³ suggested an ultrasonic agitation of EDTA/NaOCl irrigant to improve bonding of fibre posts to root canal dentin. Till date, to our knowledge there is no conclusive remark on effect of sonic energy in post space irrigation. Therefore, in the current study Endoactivator was used for activation of irrigants for PSI in Group I.

The luting of fibre posts with the three different adhesive systems i.e. Clearfill SE and Clearfill luting cement, Panavia 2.0 and Prime and Bond NT and Calibra cement was done according to manufacturer's manual. Considering the various contemporary

adhesive luting systems available in the market for bonding fibre post to root canal dentin, **Monticelli et al.**⁷⁴ 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.

Dental adhesives used in bonding to root canal dentin are predominantly used for fibre posts. For this clinical application, resin-based luting cements are used along with resin adhesives. Dental adhesives are broadly classified into "etch-and-rinse" and "self-etch" types. The etch and rinse adhesive applies a separate etching procedure to demineralize the superficial dentin surface. This is followed by priming and adhesive applications that are done either separately or in a combined form. A self-etch adhesive does not involve the etching procedure, instead it employs an acidic resin monomer to demineralize and prime dentine simultaneously. This is followed by a separate adhesive application in the 2-step self-etch adhesive. The most simplified version of this dental adhesive combines the two steps of self-etch adhesive into one, so that just one application of adhesive will perform etching, priming and adhesive coating.

Goracci et al.³⁶ demonstrated that etch-and-rinse adhesives produced significantly better bond, when compared to self-etch adhesives in bonding fibre post to root canal dentin with a resin cement. Thus, in the current research, two self etch systems have been compared with a gold standard total etch system.

Studies on the morphology of root canal dentin showed variations in the structure like accessory root canals, areas of resorption, embedded and free pulp stones, and varying amounts of irregular secondary dentine. Some areas were devoid of dentinal tubules (**Mjör et al. 2001**²⁹). These irregular features lead to different

requirements for bonding to root canal dentin compared with coronal dentin. As the number of tubules decreases from the crown to the apex (**Carrigan et al. 1984**⁷⁵), the response to acid etching and, consequently, dentine bonding can vary among different areas of the same root canal (**Ferrari et al. 2000**¹⁶). Hence, 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.

Several microscopy techniques are currently used to evaluate the resin dentin interface (RDI), including stereomicroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and confocal laser scanning microscopy (CLSM). In comparison to conventional SEM, CLSM has the advantage of providing detailed information about the presence and distribution of dental adhesives inside dentinal tubules in the total circumference of the root canal walls at relative low magnification as 100× through the use of fluorescent Rhodamine–marked primers or bonding agents. Use of fluorescent confocal microscopy for analysis of the interface of adhesive materials and tooth structure was first described by **Watson and Boyde**.²⁵ They advocated the use of fluorescent dyes, mixed into components of an adhesive system, to highlight the bonded interface.

Confocal Laser Scanning Microscopy offers improved rejection of out-of-focus noise and provides greater resolution than conventional imaging, yielding greatly enhanced images of biological structures. The intricate and often complicated methodology of specimen drying required for conventional SEM or TEM analysis, is not necessary for CLSM. This advantage leads to a decreased risk of shrinkage or desiccation artifacts and allows the same specimen to be subsequently studied using

other, additional microscopic techniques. An additional feature of the confocal principal is that it permits visualization of not only a specimen surface, but also its subsurface.²¹ Thus, in the current research CLSM was preferred over SEM and TEM and the sections were observed under the confocal laser scanning microscope.

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

In the present study, hybrid layer and resin tags were compared between different adhesive luting systems, at different regions of tooth. Also, the effect of active and passive irrigation of post space on hybrid layer and resin tags was evaluated.

The results of the study are discussed under the following headings:

1. Effect of Post Space irrigation protocol:

The maximum thickness of hybrid layer (6.40 +/- 0.75) and no. of resin tags (16.9 +/- 1.62) were observed in Active Irrigation group as compared to Passive irrigation group, irrespective of the adhesive luting system being a total etch or self etch. A highly significant difference in thickness of hybrid layer and no. of resin tags were observed between Active Irrigation and Passsive Irrigation (p value <0.001) at coronal, middle and apical levels.

According to **Cinzia S et al**⁷⁶, the action of the drills used to remove the root filling material 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.

The use of Sonic activation reduces debris and produces turbulence in the liquid leading to an increase in hydrostatic pressure, and increases in temperature and pressure, which results in shock waves on the canal walls producing the removal of detritus.

At every section, active counterparts of adhesive luting systems showed better hybridization. Thus the results obtained in the study confirmed that the activation by endoactivator had a positive effect on smear layer removal and thus formation of more no. of resin tags and a distinct hybrid layer.

These results are in accordance with study done by **Giuseppe et al**⁴⁶ who concluded that the protocols that used activated EDTA alone or in association with NaOCl were the most effective in producing a cleaner post space and thus provide better bonding surface for luting of fibre posts.

The results are also in accordance with what is shown by **Plotino et al.**⁷⁷ according to which the sonic vibration would increase the effectiveness of irrigating solutions in removing debris and thus improving the bonding process.

Since for both the groups similar irrigants in same volume and concentration were used, the effect of irrigants on smear layer removal for both active and passive irrigation groups can be considered equal.

Also better results were obtained at coronal and middle thirds as compared to apical thirds which may be due to the fact that larger canal diameters in the coronal and middle third exposes the dentin to a higher volume of irrigants. Simultaneously it facilitates the sonic activation of the irrigant, allowing a better flow of solution and

hence, improving the efficacy of smear layer removal and better penetration of resin monomers.

2. Effect of different adhesive luting systems:

Higher values for thickness of hybrid layer and no. of resin tags were obtained for groups in which Prime and Bond NT was used as compared to those where fibre posts were luted with Clearfill SE and Panavia 2.0, irrespective of the irrigation protocol used.

A highly significant difference in thickness of hybrid layer and no. of resin tags were observed between total etch and self etch adhesives used (p value <0.001) at coronal, middle and apical levels whereas a non significant difference in thickness of hybrid layer and no. of resin tags were observed between the two self etch adhesives (Panavia and Clearfill SE) (p value = 0.32)

Better results for Prime and Bond NT were seen which could be justified as phosphoric acid used for total-etch adhesives has pH=0.1-0.4, while the acidity of self-etch adhesives ranges from $\text{pH} \leq 1$ to $\text{pH} \approx 2$. Accordingly, dentin demineralization is more evident after the application of phosphoric acid because it completely removes the smear layer and opens dentinal tubules.⁷⁸

The composition of adhesives and techniques of their application are also very important. Prime & Bond NT contains acetone as a solvent. The use of acetone as a solvent in an adhesive is derived from the fact that the co-monomers of the adhesive do not dissolve in water. The role of dimethacrylate in the composition of the adhesive is to enhance crosslinking of polymers. The Prime and Bond NT also contains a mixture of the primers (i.e. HEMA) and monomers of the adhesive (i.e. bis-GMA) in a solvent

containing a low amount of water. These are applied in two layers. The first layer acts as a primer, and the second acts as an adhesive layer.

A well-formed hybrid layer and long resin tags with large number of lateral branches are characteristics of these adhesives with organic solvent: acetone.⁷⁹ The organic solvent in the composition of adhesives dehydrates acid etched dentin chemically. This leads to the lateral shrinkage of the collagen fibrils, resulting in an increase in the width of interfibrillar spaces and reduction of the hydrophilicity of collagen matrix. Lateral dentinal tubules get filled with organic solvent and the bonding agent easily penetrates into them. In this way, a very well-formed hybrid layer with long resin tags and an expressed lateral branching is obtained.⁷⁸

This is in accordance to **Scott and Thomlinson**⁸⁰, they demonstrated that the organic solvents (ethanol, acetone) remove the watery gel of glycosaminoglycans, thus removing them from the connective tissue. The collagen fibrils in the acid-etched dentin shrink laterally after the application of an adhesive with organic solvents, and the lateral dentinal tubules are filled with an organic solvent (acetone, alcohol), and adhesive resin penetrates easily into them. This is seen as perfectly formed hybrid layer and resin tags.

The decrease in thickness of hybrid layer in self-etch adhesives can be explained with their weaker acidity, which results in less demineralization of dentin, and exposes a small number of dentinal tubules, leading to poorer penetration of adhesive into dentin.⁸¹ Self-etching adhesive clearfill SE and panavia show pH=1.5 and are mild self-etch adhesives (pH between 1 and 2). They have an interaction depth between 1 and 2 μm only.⁸²

Mild self-etch adhesives are not efficient enough to dissolve the smear plugs, which close dentinal tubules, and therefore they remain in dentinal tubules as a part of a hybrid complex. That hybrid complex is characterized by a thinner hybrid layer and less resin tags.

This is in accordance with a research done by **Van Meerbeek et al.**⁸³ in **2003** and **Inoue et al**⁸⁴ in **2001** who reported that the exchange intensity induced by etch and rinse adhesives exceeds that of self-etch adhesives. This could explain the small hybrid layer thickness of the self-etching adhesives observed in the present investigation.

A previously published research evaluated the microleakage of root filled teeth restored with fibre posts luted with different resin cements.¹⁷ Teeth restored with a total etch dental adhesive including conditioning of the root canal dentin with phosphoric acid leaked significantly less than those restored with a self-etching primer. The reason for this might be that the multiple-stage adhesive system was able to produce a more uniform and thick resin–dentin interdiffusion zone than the self-etching primer. This therefore underlines the importance of a complete and deep infiltration of the adhesive system into the root canal dentin.

The results of the present 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 et al**¹⁶, 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 et al.**²⁹ also reported a decreased number of dentinal tubules from about 40,000 per mm² in the coronal region of the root canal to 14,400 per mm² in the apical region. The authors concluded that the hybrid layer would be more important 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 variation that impact the resin penetration in an apical direction. The diameter of the dentinal tubules are also larger cervically than apically (**Marion et al. 1991**⁸⁵) as well as sclerotic processes, can hamper the access to the dentinal tubules (**Mjor 1985**⁸⁶, **Wang & Weiner 1998**⁸⁷).

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 (**D’Arcangelo et al. 2008**⁸⁸). 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 et al.**³³

Thus the hypothesis of this study that there would be no differences in the penetrating ability of the used adhesive systems was rejected. The hybrid layer thickness and the number of resin tags of the bonding agents differed significantly amongst the three groups. A complete and deep infiltration of the adhesive system into root canal dentin with numerous resin tags, which has been shown for the adhesive systems ‘Prime and Bond’, is more likely to predict a durable bond of the post to the root canal dentin than the other systems.

Therefore, within the limitations of the study it can be concluded that significantly better hybrid layer and resin tags are formed when the post space is actively irrigated with EDTA and NaOCl and when fibre post is luted with a total etch system.

LIMITATIONS

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1. Extracted teeth show great degree of variation, though all care was taken to handle and store them according to standard guidelines.
2. Exact simulation of the oral conditions was not possible as this was an in vitro study. Therefore, the results cannot be directly extrapolated to the clinical situation.
3. The study only evaluated the morphological aspect of RDI. Further correlation of these morphological aspect with bond strength may establish the validity of claim.

SUMMARY AND CONCLUSION

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In the past decade, foundation materials chosen for restoring endodontically treated teeth have changed from exclusively rigid materials (metal and zirconium posts) to materials having mechanical characteristics similar to that of dentin (fibre posts and composite resin). Fibre posts are chemically compatible with Bis-GMA based luting agents commonly used in bonding procedures and they can be cemented using an adhesive technique. However, prospective clinical studies have evaluated the efficacy of fibre posts and identified debonding along the resin dentin interface as the most frequent mode of failure.

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

The present in vitro study was carried out to evaluate the hybrid layer and resin tags at resin dentin interface in adhesive resin luting systems and the effect of active and passive irrigation on them using a confocal laser scanning microscope.

120 freshly extracted human maxillary anteriors, which fulfilled the inclusion criteria were selected for the study. 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 two groups of 60 each:

Group I: Active Irrigation of Post Space

Group II: Passive Irrigation of Post Space

The teeth were further divided into three sub groups according to the different adhesive luting system used:

Sub Group A: Clearfill SE Bond and Clearfill luting cement

Sub Group B: Panavia

Sub Group C: Prime and Bond NT and Calibra Resin cement.

Fibre posts were tried and luted using the respective 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.

The results obtained indicated that there was a highly significant difference in thickness of hybrid layer and no. of resin tags in groups actively irrigated when compared with those passively irrigated at coronal, middle and apical thirds (p-value <0.001).

It was also found that a highly significant difference existed in thickness of hybrid layer and no. of resin tags when the three different adhesive luting systems were compared.

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

1. A uniform hybrid layer and continuous resin tags were seen in all groups that were actively irrigated.
2. An active irrigation protocol of the post space resulted in a significant increase in the thickness of hybrid layer and no. of resin tags.
3. A significantly thicker hybrid layer and higher no. of resin tags were obtained when the fibre posts were luted with a total etch system.
4. The thickness of hybrid layer and no. of resin tags 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 and higher no. of resin tags are formed when the post space is actively irrigated and when the fibre post is luted with a total etch system. However a correlative study with bond strength tests could give a conclusive remark on the effect of post space irrigation and adhesive luting systems on hybrid layer and no. of resin tags and their role in bonding of fibre posts to root canal dentin.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Monga P, Sharma V, Kumar S. Comparison of fracture resistance of endodontically treated teeth using different coronal restorative materials: an in vitro study. *J Conserv Dent* 2009;12(4):154-159.
2. Cheung W. A review of the management of endodontically treated teeth. Post, core and the final restoration. *J Am Dent Assoc.* 2005;136(5):611-9.
3. Gonzaga C. Restoration of endodontically treated teeth. *RSBO* 2011;8(3): 33-46.
4. Sorensen JA, Engelman MJ. Effect of post adaptation on fracture resistance of endodontically treated teeth. *J Prosthet Dent* 1990;64(4):419-24.

5. Boschian Pest L, Cavalli G. Adhesive post endodontic restorations with fibre posts: push-out tests and SEM observations. *Dent Mater* 2002;18(8):596-602.
6. Asmussen E, Peutzfeldt A, Heitmann T. Stiffness, elastic limit and strength of newer types of endodontic posts. *J Dent* 1999;27(4):275-8.
7. Feilzer AJ. Setting stresses in composites for two different curing modes. *Dent Mater* 1993;9(1):2-5.
8. Carvalho RM, Pereira JC. A review of polymerization contraction: the influence of stress development versus stress relief. *Oper Dent* 1996;21(1):17-24
9. Tay FR, Loushine RJ, Lambrechts P, Weller RN, Pashley DH. Geometric factors affecting dentin bonding in root canals: a theoretical modeling approach. *J Endod* 2005;31(8):584–9.
10. Bouillaguet S, Troesch S, Wataha JC, Krejci I, Meyer JM, Pashley DH. Microtensile bond strength between adhesive cements and root canal dentin. *Dent Mater* 2003;19(3):199–205.
11. Roberts HW, Leonard DL, Vandewalle KS, Cohen ME. The effect of a translucent post on resin composite depth of cure. *Dent Mater* 2004;20(7):617-22.
12. Nakabayashi N, Nakamura M, Yasuda N. Hybrid layer as a dentin-bonding mechanism. *J Esthet Dent* 1991;3(4):133–8.
13. Titley K, Chernecky R, Chan A, Smith D. The composition and ultrastructure

- of resin tags in etched dentin. *Am J Dent* 1995;8(5):224–30.
14. Ferrari M, Davidson CL. In vivo resin-dentin interdiffusion and tag formation with lateral branches of two adhesive systems. *J Prosthet Dent* 1996;76(3):250–3.
 15. Pegoretti A, Fambri L, Zappini G, Bianchetti M. Finite element analysis of a glass fibre reinforced composite endodontic post. *Biomaterials* 2002;23(13):2667–82.
 16. Ferrari M, Vichi A, Mannocci F, Mason PN. Retrospective study of the clinical performance of fiber posts. *Am J Dent* 2000;13,9B–13B.
 17. Mannocci F, Bertelli E, Watson TF, Ford TP. Resin- dentin interfaces of endodontically-treated restored teeth. *Am J Dent* 2003;16(1):28–32.
 18. Naumann M, Koelpin M, Beuer F, Meyer-Lueckel H. 10-year survival evaluation for glass-fiber-supported postendodontic restoration: a prospective observational clinical study. *J Endod* 2012;38(4):432–435.
 19. Bitter K, Paris S. A confocal laser scanning microscope investigation of different dental adhesives bonded to root canal dentin. *Int Endod J* 2004;37(12):840-8.
 20. PashleyDH, CiucchiB, SanoH. Permeability of dentin to adhesive agents. *Quintessence Int* 1993;24(9):618–31.
 21. Pioch T. Novel feasibilities for visualizing the contact zone between dentin and resin by application of Leica CLSM. *Leica Sci Tech Infor.* 1996;11:80–83.

22. Goldman LB, Goldman M, Kronman JH, Lin PS. The efficacy of several irrigating solutions for endodontics: a scanning electron microscopic study. *Oral Surg Oral Med Oral Pathol* 1981;52(2):197–204.
23. Nakabayashi N, Kojima K, Masuhara E. The promotion of adhesion by infiltration of monomers into tooth substrates. *J Biomed Mater Res* 1982; 16(3):265-73.
24. Hiltner RS, Kulild JC, Weller RN. Effect of mechanical versus thermal removal of gutta-percha on the quality of the apical seal following post space preparation. *J Endod* 1992;18(9):451-454
25. Watson TF, Boyde A. Tandem scanning reflected light microscopy: applications in clinical dental research. *Scanning Microsc* 1987;1(4):1971–81.
26. Czonstkowsky M, Wilson EG, Holstein FA. The smear layer in endodontics. *Dent Clin North Am* 1990;34(1):13– 25.
27. Van Meerbeek B. Morphological aspects of the resin dentin interdiffusion zone with different dentin adhesive systems. *J Dent Res* 1992;71(8):1530-40.
28. Yoshiyama M, Matsuo T, Ebisu S, Pashley D. Regional bond strengths of self-etching/self-priming adhesive systems. *J Dent* 1996;24(6):435-42.
29. Mjor A, Smith M, Ferrari M, Mannocci F. The structure of dentine in the apical region of human teeth. *Int Endod J* 2000;34(5):346-53.
30. Mayhew JT, Windchy AM, Goldsmith LJ, Gettleman L. Effect of root canal sealers and irrigation agents on retention of preformed posts luted with a resin cement. *J Endod* 2000;26(6):341-4.

31. Ngoh EC, Pashley DH, Loushine RJ, Weller RN, Kimbrough WF. Effects of eugenol on resin bond strengths to root canal dentine. *J Endod* 2001;27(6):411-4.
32. Calt S, Serper A. Time-dependent effects of EDTA on dentin structures. *J Endod* 2002;28(1):17-9
33. Ferrari M, Grandini S, Simonetti M. Influence of a microbrush on bonding fibre post into root canals under clinical conditions. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2002;94(5):627-31.
34. Serafino C, Gallina G, Cumbo E, Ferrari M, Siena and Palermo. Surface debris of canal walls after post space preparation in endodontically treated teeth: A scanning electron microscopic study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2004;97(3):381-7.
35. Luz MA, Chavez VE, Netto NG. Scanning electron microscopic examination of 3 different adhesive systems. *Quintessence Int* 2005;36(9):687-94.
36. Goracci C, Sadek FT, Fabianelli A, et al. Evaluation of the adhesion of fiber posts to intraradicular dentin. *Oper Dent* 2005;30(5):627-35.
37. Garcia-Godoy F, Loushine RJ, Itthagarun A, Weller RN, Murray PE, Feilzer AJ, Pashley DH, Tay FR. Application of biologically-oriented dentine bonding principles to the use of endodontic irrigants. *Am J Dent* 2005;18(6):281-90.
38. Hayashi M, Takahashi Y, Imazato S, Ebisu S. Fracture resistance of pulpless teeth restored with post-cores and crowns. *Dent Mater* 2006;22(5):477-85.
39. Menezes MS, Queiroz EC, Campos RE, Martins LRM, Soares CJ. Influence of

- endodontic sealer cement on fibre glass post bond strength to root dentine. *Int Endod J* 2008;41(6):476–484.
40. Gu XH, Mao CY, Kern M. Effect of different irrigation on smear layer removal after post space preparation. *J Endod* 2009;35(4):583-586.
41. Malyk Y, Kaaden C, Hickel R, Llie N. Analysis of resin tags formation in root canal dentin: a cross sectional study. *Int Endod J* 2010;43(1):47-56.
42. Vilanova WV, Carvalho-Junior JR, Alfredo E, Sousa-Neto MD, Silva-Sousa YT. Effect of intracanal irrigants on the bond strength of epoxy resin-based and methacrylate resin-based sealers to root canal walls. *Int Endod J* 2012;45:42-8.
43. Marigo L, Lajolo C, Castagnola R, Angerame, Somma. Morphological confocal laser scanning microscope evaluation of four different “etch and rinse” adhesives in post endodontic restoration. *Dent Mater J* 2012;31(6):988-994.
44. Srirekha A, Rashmi K, Hegde J, Lekha S. An In Vitro Evaluation of Passive Ultrasonic Agitation of Different Irrigants on Smear Layer Removal After Post Space Preparation: A Scanning Electron Microscopic Study. *J Indian Prosthodont Soc* 2013;13(3):240–246
45. Ekambaram M. Bonding of resin adhesives to root canal dentin. *Int J Adhes Adhesi* 2015;61:23-34
46. Giudice G, Lizio A, Giudice R, Centofanti A, Rizzo G. The Effect of Different Cleaning Protocols on Post Space: A SEM Study. *Int J Dent* 2016;
47. Awad M. Assessment of resin-dentin interfacial morphology of two ethanol-based universal adhesives: A scanning electron microscopy study. *Eur J Dent*

- 2017;11(2):206-209.
48. Carvalho M, Morari V, Susin A, Rocha R. Endodontic irrigation protocol: Effects on bonding of adhesive systems to coronal enamel and dentin. *J Esthet Restor Dent*. 2017;29(3):222-228.
49. Ferreira JC, Pires PT, Silva MJ. Morphology of the dentin resin interface yielded by two step etch and rinse adhesive with different solvents. *J Contemp Dent Pract* 2017;18(10):947-958.
50. Kohn WG, Collins AS, Cleveland JL, Harte JA, Eklund KJ, Malvitz DM. Centre for Disease Control and Prevention (CDC). Guidelines for Infection Control in Dental Health Care Settings. *MMWR Recomm Rep* 2003;52(RR-17):1-61.
51. Jameson MW, Tidmarsh BG, Hood JA. Effect of storage media on subsequent water loss and regain by human and bovine dentine and on mechanical properties of human dentine in vitro. *Arch Oral Biol* 1994;39(9):759-67.
52. Gillen BM, Looney SW, Gu LS, Loushine BA, Weller RN, Loushine RJ, Pashley DH, Tay FR. Impact of the quality of coronal restoration versus the quality of root canal fillings on success of root canal treatment: a systematic review and meta-analysis. *J Endod* 2011;37(7):895-902.
53. Ray HA, Trope M. Periapical status of endodontically treated teeth in relation to the technical quality of the root filling and the coronal restoration. *Int Endod J* 1995;28(1):12-8.
54. Safavi KE, Dowden WE, Langeland K. Influence of delayed coronal permanent restoration on endodontic prognosis. *Endod Dent Traumatol* 1987;3(4):187-91.

55. Marshall FJ, Massler M. The sealing of pulpless teeth evaluated with radioisotopes. *J Dent Med* 1961;16:L172-84.
56. Shillingburg, H.T., Jr., Kessler, J.C. Restoration of the endodontically treated tooth; Quintessence Publishing Co., Inc.: Chicago, Berlin, Rio de Janeiro, Tokyo, 1982.
57. Rosenstiel, S.F., Land, M.F., Fujimoto, J. Contemporary fixed prosthodontics, 2nd; Mosby: St. Louis, Baltimore, Boston, Chicago, London, Philadelphia, Sydney, Toronto, 1995.
58. Torbjörner A, Karlsson S, Syverud M, Hensten-Pettersen A. Carbon fiber reinforced root canal posts. Mechanical and cytotoxic properties. *Eur J Oral Sci.* 1996;104(5-6):605-11.
59. Nakabayashi N, Ashizawa M, Nakamura M. Identification of a resin–dentin hybrid layer in vital human dentin created in vivo: durable bonding to vital dentin. *Quintessence Int* 1992;23(2),135–41.
60. Walshaw PR, McComb D. SEM evaluation of the resin–dentin interface with proprietary bonding agents in human subjects. *J Dent Res* 1994;73(5), 1079–87.
61. Mannocci F, Ferrari M, Watson TF. Mikroleakage of endodontically treated teeth restored with fibre posts and composite cores after cyclic loading: a confocal microscopic study. *J Prosthet Dent* 2001;85(3):284-91.

62. Goyal N. Traumatic dental injuries prevalence and their impact on self esteem among adolescents in India: a comparative study. *J Clin Diagn Res* 2017;11(8):106-110.
63. Shanmugaraj M, Nivedha R, Mathan R, Balagopal S. Evaluation of working length determination methods: an in vivo / ex vivo study. *Indian J Dent Res.* 2007;18(2):60-2
64. Vertucci F. Root canal morphology and its relationship to endodontic procedures. *Endod Topics* 2005;10(1):3-29.
65. Demiryürek EO, Külünk S, Yüksel G, Saraç D, Bulucu B. Effects of three canal sealers on bond strength of a fibre post. *J Endod* 2010;36:497-501.
66. Teixeira CS, Pasternak-Junior B, Borges AH, Paulino SM, Sousa-Neto MD. Influence of endodontic sealers on the bond strength of carbon fibre posts. *J Biomed Mater Res B Appl Biomater* 2008;84:430-5.
67. Schwartz RS, Murchison DF, Walker WA 3rd. Effects of eugenol and noneugenol endodontic sealer cements on post retention. *J Endod* 1998;24:564-7.
68. Wu MK, Ozok AR. Sealer distribution in root canals obturated by three techniques. *Int Endod J* 2000;33(4):340-5.
69. Coniglio I, Magni E, Gorracci. Post space cleaning using a new nickel titanium endodontic drill combined with different cleaning regimes. *J Endod* 2008;34(1):83-6
70. Ari H, Yasınar E, Belli S. Effects of NaOCl on bond strengths of resin cements to root canal dentin. *J Endod* 2003; 29(4):248–51.

71. Erdemir A, Ari H, Güngönes □ H, Belli S. Effect of medications for root canal treatment on bonding to root canal dentin. *J Endod* 2004;30(2):113–6.
72. Lai SC, Mak YF, Cheung GS et al. Reversal of compromised bonding to oxidized etched dentin. *J Dent Res* 2001; 80(10):1919–24.
73. Zhang L, Huang L, Xiong Y, Fang M, Chen JH, Ferrari M. Effect of post-space treatment on retention of fiber posts in different root regions using two self-etching systems. *Eur J Oral Sci* 2008; 116: 280–6.
74. Monticelli F, Osorio R, Albaladejo A, Aguilera FS, Ferrari M, Tay FR, Toledano M.J Effects of adhesive systems and luting agents on bonding of fiber posts to root canal dentin. *J Biomed Mater Res B Appl Biomater* 2006;77:195-200.
75. Carrigan PJ, Morse DR, Furst ML, Sinai IH. A scanning electron microscopic evaluation of human dentinal tubules according to age and location. *J Endod* 1984;10, 359–63.
76. Cinzia S, Giuseppe G, Enzo C, Ferrari. Surface debris of canal walls after post space preparation in endodontically treated teeth: an SEM study. *Oral Surg Oral Med Oral Pathol* 2004;97:381–387.
77. Plotino, G.; Pameijer, C. H.; Grande, N. M. & Somma, F. Ultrasonics in endodontics: a review of the literature. *J. Endod* 2007;33(2):81-95..
78. Pashley DH, Tay FR, Breschi L, Tjäderhane L, Carvalho RM, Carrilho M, et al. State of the art etch-and-rinse adhesives. *Dent Mater* 2011;27:1-16.
79. Sauro S, Watson TF, Manocci F, Miyake K, Huffman BP, Tay FR, et al. Two-photon laser confocal microscopy of micro- permeability of resin-dentin bonds

- made with water or ethanol wet bonding. *J Biomed Mater Res Part B Appl Biomater.* 2009;90B:327-37.
80. Scott JE, Thomlinson AM. The structure of interfibrillar proteoglycans bridges (shape modules) in extracellular matrix of fibrous connective tissues and their stability in various chemical environments. *J Anat.* 1998;192:391-405.
81. Lee KW, Son HH, Yoshiyama M, Tay FR, Carvalho RM, Pashley DH. Sealing properties of a self-etching primer system to normal caries-affected dentin and caries-infected dentin. *Am J Dent* 2003;16:68A-72A.
82. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt KL. State of the art of self-etch adhesives. *Dent Mater.* 2011;27:17-28.
83. Van Meerbeek B, De Munck J, Yoshida Y .Buonocore memorial lecture. Adhesion to enamel and dentin: current status and future challenges. *Oper Dent* 2003;28:215–35.
84. Inoue S, Vargas MA, Abe Y. Microtensile bond strength of eleven contemporary adhesives to dentin. *J Adhes Dent* 2001;3(3):237–45.
85. Marion D, Jean A, Hamel H, Kerebel LM, Kerebel B. Scanning electron microscopic study of odontoblasts and circumpulpal dentin in a human tooth. *Oral Surg Oral Med Oral Path Oral Radio Endod* 1991;72:473–8.
86. Mjor IA. Dentin-predentin complex and its permeability: pathology and treatment overview. *J Dent Res*;64:621–7.
87. Wang RZ, Weiner S. Strain-structure relations in human teeth using Moire fringes. *J Biomech* 1998;31(2):135–41.

88. D'Arcangelo C, Zazzeroni S, D'Amario M. Bond strengths of three types of fibre-reinforced post systems in various regions of root canals. *Int Endod J* 2008;41(4):322–8.

TABLES AND GRAPHS

Table 1: Descriptive Statistics i.e Mean and Standard Deviation for thickness of hybrid layer according to groups (Active Irrigation and Passive Irrigation) and subgroups (Clearfill SE, Panavia, Prime and bond NT) at Coronal, Middle and Apical sections.

HYBRID LAYER (µm)			Group					
			Active (n=60)			Passive (n=60)		
			Subgroup			Subgroup		
			Clearfill SE (n=20)	Panavia (n=20)	Prime & Bond (n=20)	Clearfill SE (n=20)	Panavia (n=20)	Prime & Bond (n=20)
Section	Coronal	Mean	5.36	4.19	6.40	3.20	2.10	5.35
		SD	0.53	0.69	0.75	0.70	0.45	0.59
	Middle	Mean	4.30	4.05	6.35	2.20	2.05	4.80
		SD	0.66	0.76	0.67	1.01	0.69	0.95
	Apical	Mean	3.45	3.35	5.85	2.05	1.70	4.15
		SD	0.60	0.59	0.59	0.83	0.66	1.04

Table 2: Comparison between groups and subgroups and their effect on thickness of hybrid layer at CORONAL section using two-way analysis of variance

Source	SS	DF	MSS	F	P-value
Corrected Model	251.911 ^a	5	50.382	128.158	< 0.0001*
Intercept	2358.356	1	2358.356	5998.974	< 0.0001*
Group	93.598	1	93.598	238.086	< 0.0001*
Subgroup	150.588	2	75.294	191.527	< 0.0001*
Group * Subgroup	7.724	2	3.862	9.824	< 0.0001*
Error	44.816	114	.393		
Total	2655.083	120			

^a R Squared = .849 (Adjusted R Squared = .842); *Highly significant

Table 2a: Comparison of main effect of Irrigation Group on thickness of hybrid layer at CORONAL section as per subgroups

Subgroup		Sum of Squares	DF	Mean Square	F	P-value
Clearfill SE	Contrast	46.721	1	46.721	118.844	< 0.0001*
	Error	44.816	114	.393		
Panavia	Contrast	43.577	1	43.577	110.846	< 0.0001*
	Error	44.816	114	.393		
Prime & Bond	Contrast	11.025	1	11.025	28.044	< 0.0001*
	Error	44.816	114	.393		

*Highly significant

Table 2b: Comparison of main effect of subgroups on thickness of hybrid layer at CORONAL section as per groups

Group		Sum of Squares	DF	Mean Square	F	P-value
Active	Contrast	49.013	2	24.506	62.337	< 0.0001*
	Error	44.816	114	.393		
Passive	Contrast	109.300	2	54.650	139.014	< 0.0001*
	Error	44.816	114	.393		

*Highly significant

Table 3: Comparison between groups and subgroups and their effect on thickness of hybrid layer at MIDDLE section using two-way analysis of variance

Source	SS	DF	MSS	F	P-value
Corrected Model	265.742 ^a	5	53.148	82.942	< 0.0001*
Intercept	1880.208	1	1880.208	2934.206	< 0.0001*
Group	106.408	1	106.408	166.058	< 0.0001*
Subgroup	157.617	2	78.808	122.986	< 0.0001*
Group * Subgroup	1.717	2	.858	1.339	0.266
Error	73.050	114	.641		
Total	2219.000	120			

^a R Squared = .784 (Adjusted R Squared = .775); *Highly significant

Table 3a: Descriptive statistics for thickness of hybrid layer at group level for MIDDLE section

Group	N	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Active	60	4.900	0.103	4.695	5.105
Passive	60	3.017	0.103	2.812	3.221

Table 3b: Descriptive statistics for thickness of hybrid layer at subgroup level for MIDDLE section

Subgroup	N	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Clearfill SE	40	3.250	0.127	2.999	3.501
Panavia	40	3.050	0.127	2.799	3.301
Prime & Bond	40	5.575	0.127	5.324	5.826

Table 3c: Paired comparison of thickness of hybrid layer between subgroups for MIDDLE section using Tukey's post-hoc test

Subgroup		Mean difference	Std. Error	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Clearfill SE	Panavia	0.2000	0.179	0.505	-0.2251	0.6251
	Prime & Bond	-2.3250	0.179	< 0.0001	-2.7501	-1.8999
Panavia	Prime & Bond	-2.5250	0.179	< 0.0001	-2.9501	-2.0999

Table 4: Comparison between groups and subgroups and their effect on thickness of hybrid layer at APICAL section using two-way analysis of variance

Source	SS	DF	MSS	F	P-value
Corrected Model	225.575 ^a	5	45.115	83.289	< 0.0001*
Intercept	1407.675	1	1407.675	2598.785	< 0.0001*
Group	75.208	1	75.208	138.846	< 0.0001*
Subgroup	149.850	2	74.925	138.323	< 0.0001*
Group * Subgroup	.517	2	.258	.477	0.622
Error	61.750	114	.542		
Total	1695.000	120			

^a R Squared = .785 (Adjusted R Squared = .776); *Highly significant

Table 4a: Descriptive statistics for thickness of hybrid layer at group level for APICAL section

Group	N	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Active	60	4.217	0.095	4.028	4.405
Passive	60	2.633	0.095	2.445	2.822

Table 4b: Descriptive statistics for thickness of hybrid layer at subgroup level for APICAL section

Subgroup	N	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Clearfill SE	40	2.750	0.116	2.519	2.981
Panavia	40	2.525	0.116	2.294	2.756
Prime & Bond	40	5.000	0.116	4.769	5.231

Table 4c: Paired comparison of thickness of hybrid layer between subgroups for APICAL section using Tukey’s post-hoc test

Subgroup		Mean difference	Std. Error	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Clearfill SE	Panavia	0.2250	0.164	0.362	-0.1658	0.6158
	Prime & Bond	-2.2500	0.164	< 0.0001	-2.6408	-1.8592
Panavia	Prime & Bond	-2.4750	0.164	< 0.0001	-2.8658	-2.0842

Table 5: Descriptive Statistics i.e Mean and Standard Deviation for no. of resin tags according to groups (Active Irrigation and Passive Irrigation) and subgroups (Clearfill SE, Panavia, Prime and bond NT) at Coronal, Middle and Apical sections

RESIN TAGS			Group					
			Active (n=60)			Passive (n=60)		
			Subgroup			Subgroup		
			Clearfill SE (n=20)	Panavia (n=20)	Prime & Bond (n=20)	Clearfill SE (n=20)	Panavia (n=20)	Prime & Bond (n=20)
Section	Coronal (n=20)	Mean	16.90	12.20	21.30	12.00	9.30	16.35
		SD	1.62	2.24	2.60	2.27	1.84	2.25
	Middle (n=20)	Mean	12.55	9.80	18.05	8.80	7.20	13.25
		SD	1.73	1.82	2.01	1.70	2.07	3.01
	Apical (n=20)	Mean	8.70	7.30	14.40	6.20	5.20	8.90
		SD	1.72	2.03	1.82	1.91	1.06	2.27

Table 6: Comparison between groups and subgroups and their effect on no. of resin tags at CORONAL section using two-way analysis of variance

Source	SS	DF	MSS	F	P-value
Corrected Model	1876.375 ^a	5	375.275	80.424	< 0.0001*
Intercept	25842.675	1	25842.675	5538.237	< 0.0001*
Group	541.875	1	541.875	116.127	< 0.0001*
Subgroup	1307.150	2	653.575	140.065	< 0.0001*
Group * Subgroup	27.350	2	13.675	2.931	0.057
Error	531.950	114	4.666		
Total	28251.000	120			

^aR Squared = .779 (Adjusted R Squared = .769); *Highly significant

Table 6a: Descriptive statistics for no. of resin tags at group level for CORONAL section

Group	N	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Active	60	16.800	0.279	16.248	17.352
Passive	60	12.550	0.279	11.998	13.102

Table 6b: Descriptive statistics for no. of resin tags at sub group level for CORONAL section

Subgroup	n	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Clearfill SE	40	14.450	0.342	13.773	15.127
Panavia	40	10.750	0.342	10.073	11.427
Prime & Bond	40	18.825	0.342	18.148	19.502

Table 6c: Paired comparison of no. of resin tags between subgroups for CORONAL section using Tukey's post-hoc test

Subgroup		Mean difference	Std. Error	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Clearfill SE	Panavia	3.700	0.483	< 0.0001*	2.5530	4.8470
	Prime & Bond	-4.375	0.483	< 0.0001*	-5.5220	-3.2280
Panavia	Prime & Bond	-8.075	0.483	< 0.0001*	-9.2220	-6.9280

*Highly significant

Table 7: Comparison between groups and subgroups and their effect on no. of resin tags at MIDDLE section using two-way analysis of variance

Source	SS	DF	MSS	F	P-value
Corrected Model	1513.342 ^a	5	302.668	68.291	< 0.0001*
Intercept	16170.408	1	16170.408	3648.543	< 0.0001*
Group	414.408	1	414.408	93.503	< 0.0001*
Subgroup	1074.717	2	537.358	121.245	< 0.0001*
Group * Subgroup	24.217	2	12.108	2.732	0.069
Error	505.250	114	4.432		
Total	18189.000	120			

^a R Squared = .750 (Adjusted R Squared = .739); *Highly significant

Table 7a: Descriptive statistics for no. of resin tags at group level for MIDDLE section

Group	N	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Active	60	13.467	0.272	12.928	14.005
Passive	60	9.750	0.272	9.212	10.288

Table 7b: Descriptive statistics for no. of resin tags at subgroup level for MIDDLE section

Subgroup	n	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Clearfill	40	10.675	0.333	10.016	11.334
Panavia	40	8.500	0.333	7.841	9.159
Calibra	40	15.650	0.333	14.991	16.309

Table 7c: Paired comparison of no. of resin tags between subgroups for MIDDLE section using Tukey's post-hoc test

Subgroup		Mean difference	Std. Error	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Clearfill	Panavia	2.1750	.470	< 0.0001	1.0571	3.2929
	Calibra	-4.9750	.470	< 0.0001	-6.0929	-3.8571
Panavia	Calibra	-7.1500	.470	< 0.0001	-8.2679	-6.0321

Table 8: Comparison between groups and subgroups and their effect on no. of resin tags at APICAL section using two-way analysis of variance

Source	SS	DF	MSS	F	P-value
Corrected Model	1052.300 ^a	5	210.460	62.253	< 0.0001*
Intercept	8568.300	1	8568.300	2534.474	< 0.0001*
Group	340.033	1	340.033	100.581	< 0.0001*
Subgroup	643.200	2	321.600	95.128	< 0.0001*
Group * Subgroup	69.067	2	34.533	10.215	< 0.0001*
Error	385.400	114	3.381		
Total	10006.000	120			

^a R Squared = .732 (Adjusted R Squared = .720); *Highly significant

Table 8a: Comparison of main effect of Irrigation Group on no. of resin tags at APICAL section as per subgroups

Subgroup		Sum of Squares	DF	Mean Square	F	P-value
Clearfill	Contrast	62.500	1	62.500	18.487	< 0.0001*
	Error	385.400	114	3.381		
Panavia	Contrast	44.100	1	44.100	13.045	< 0.0001*
	Error	385.400	114	3.381		
Calibra	Contrast	302.500	1	302.500	89.478	< 0.0001*
	Error	385.400	114	3.381		

*Highly significant

Table 8b: Comparison of main effect of subgroups on no. of resin tags at APICAL section as per groups

Group		Sum of Squares	DF	Mean Square	F	P-value
Active	Contrast	565.733	2	282.867	83.671	< 0.0001*
	Error	385.400	114	3.381		
Passive	Contrast	146.533	2	73.267	21.672	< 0.0001*
	Error	385.400	114	3.381		

*Highly significant

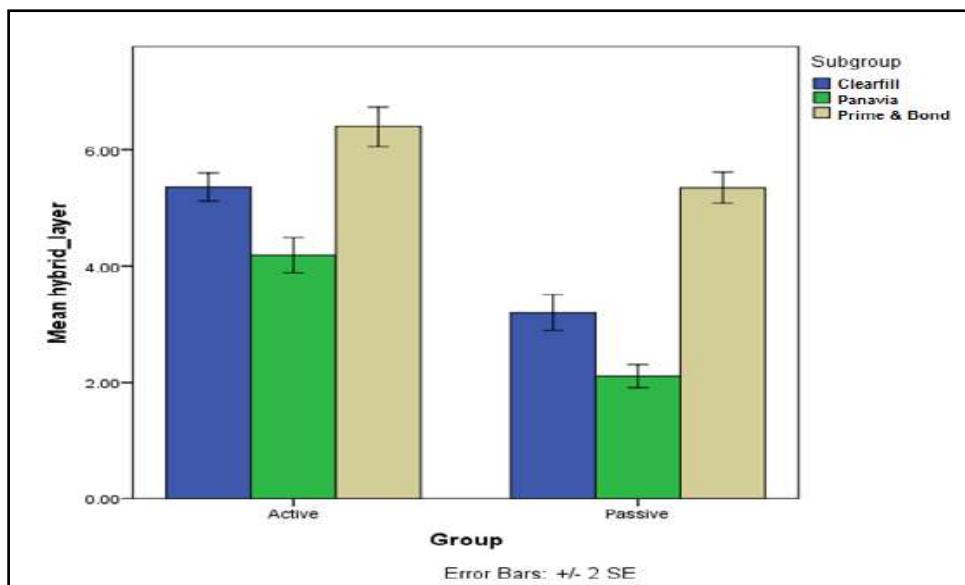


Figure 1: Bar chart with error bars showing mean thickness of hybrid layer according to groups and subgroups for CORONAL section

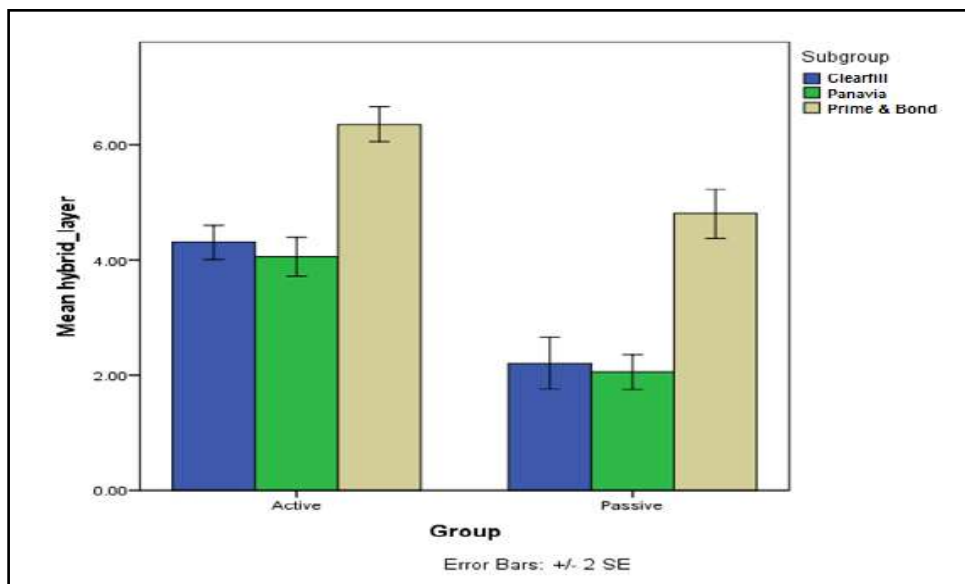


Figure 2: Bar chart with error bars showing mean thickness of hybrid layer according to groups and subgroups for MIDDLE section

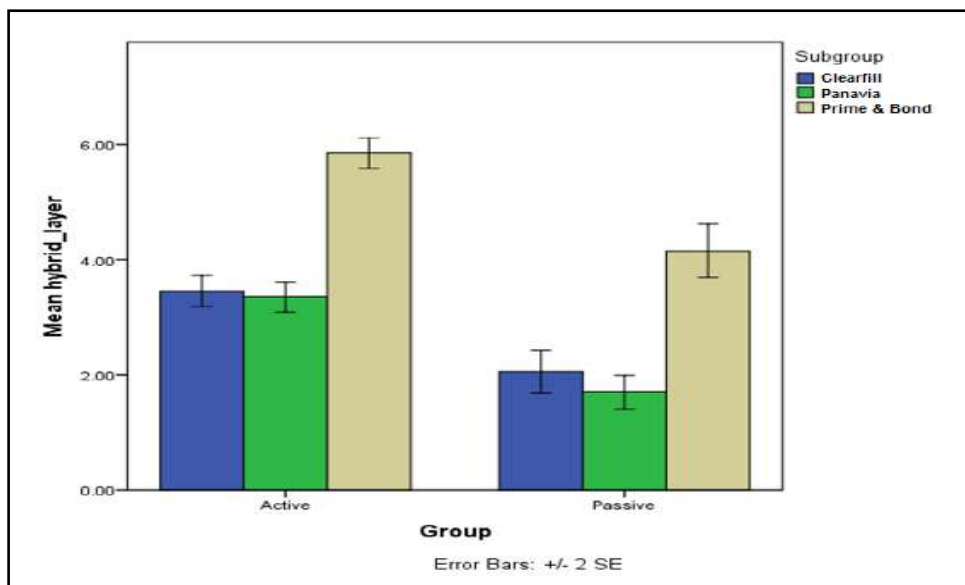


Figure 3: Bar chart with error bars showing mean thickness of hybrid layer according to groups and subgroups for APICAL section

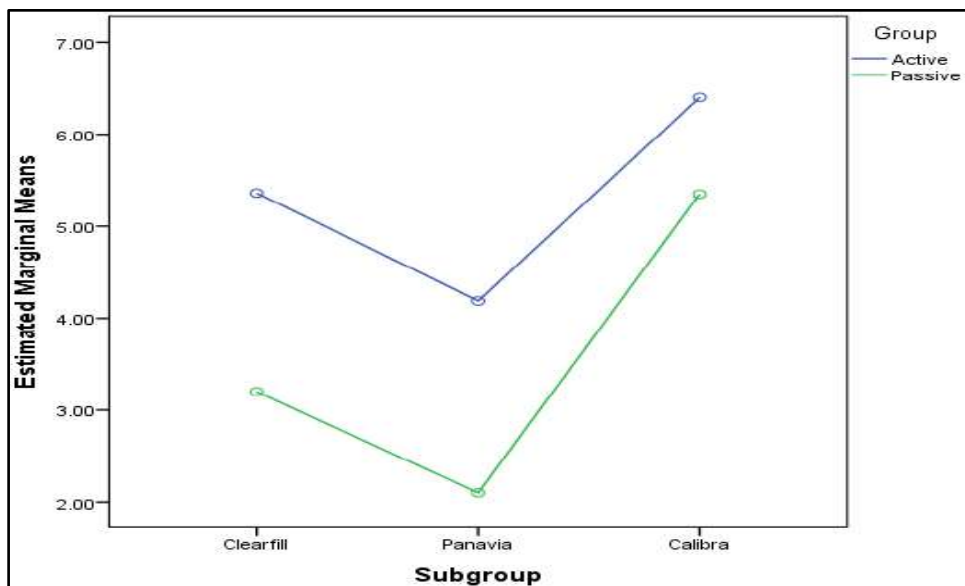


Figure 4: Mean thickness of hybrid layer for each subgroups according to groups for CORONAL section

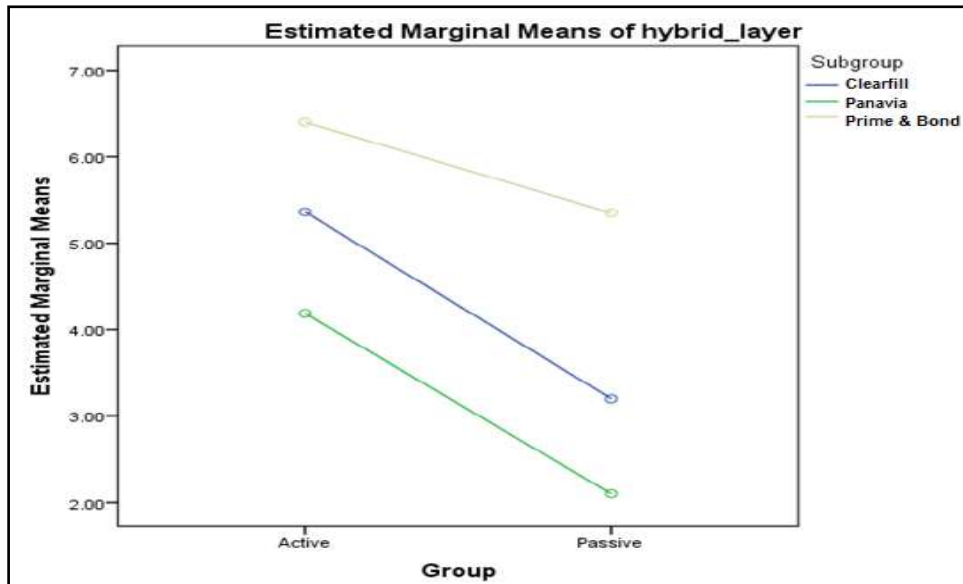


Figure 5: Mean thickness of hybrid layer for each group according to subgroups for CORONAL section

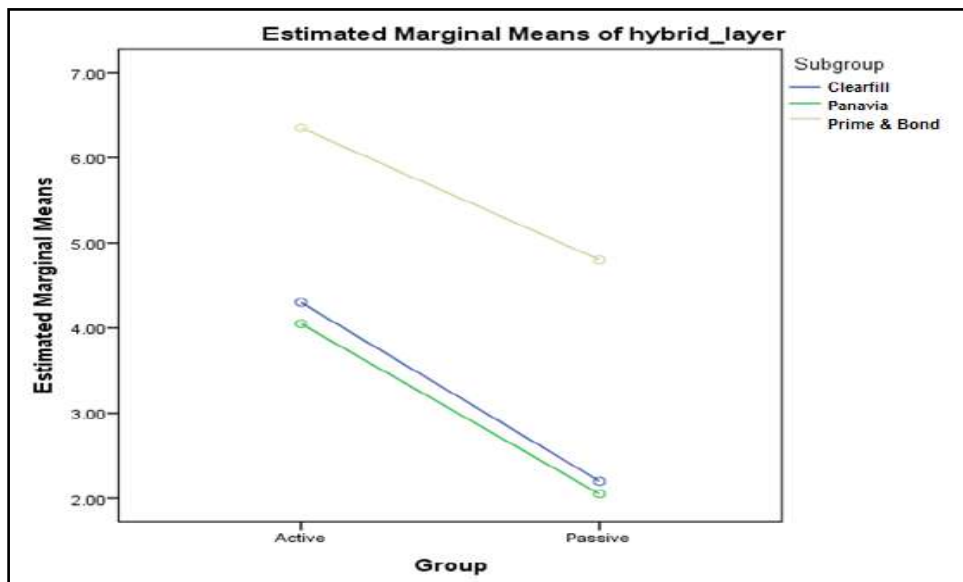


Figure 6: Mean thickness of hybrid layer for each group according to subgroups for MIDDLE section

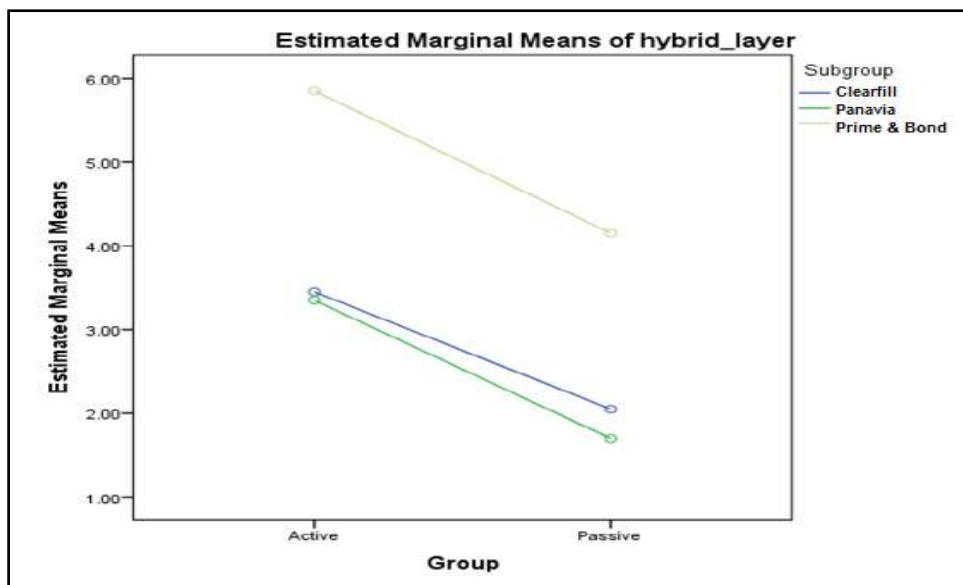


Figure 7: Mean thickness of hybrid layer for each group according to subgroups for APICAL section

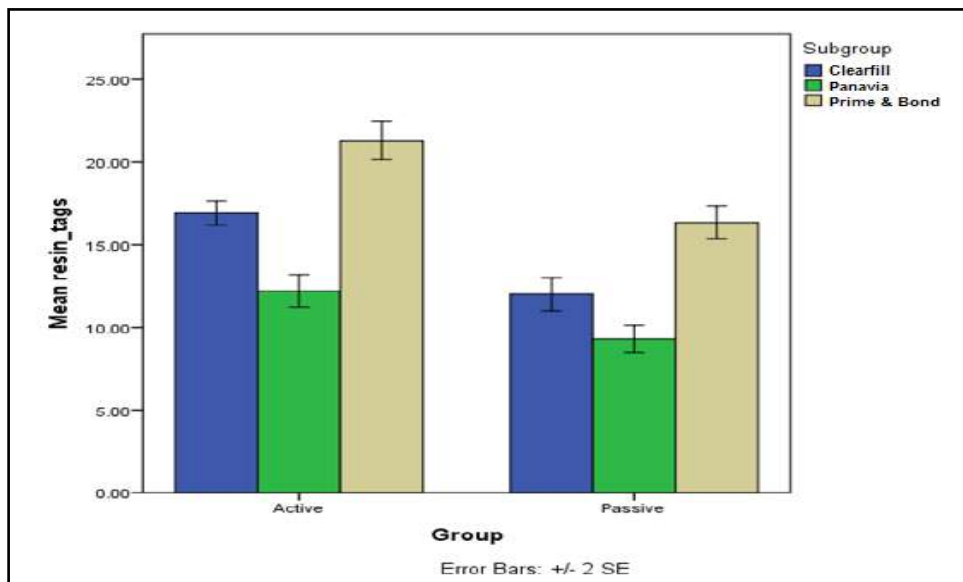


Figure 8: Bar chart with error bars showing mean number of resin tags according to groups and subgroups for CORONAL section

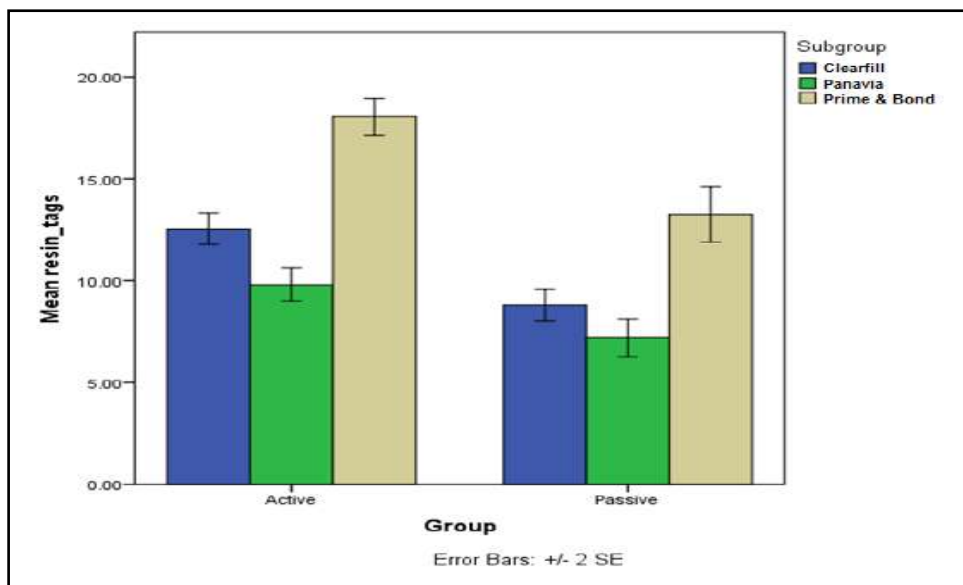


Figure 9: Bar chart with error bars showing mean number of resin tags according to groups and subgroups for MIDDLE section

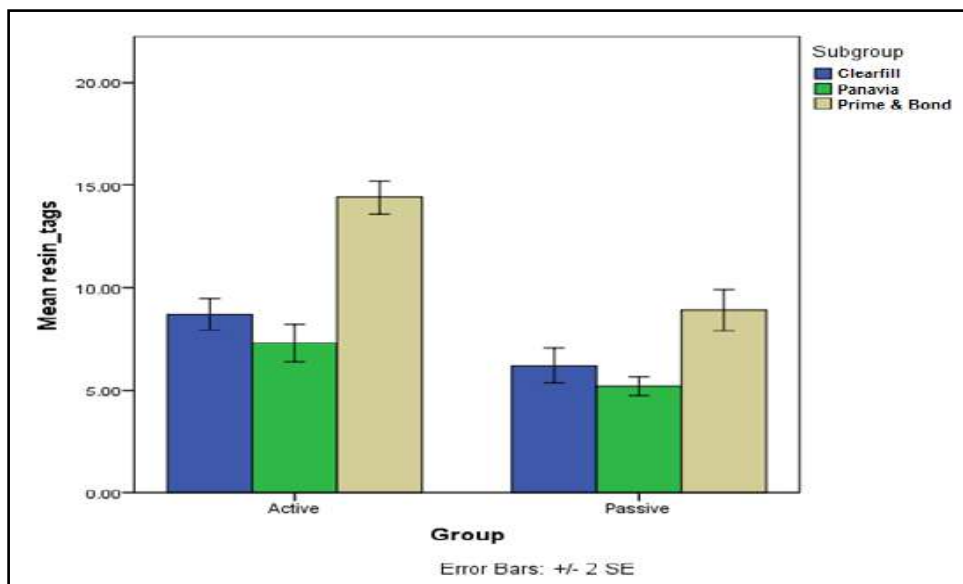


Figure 10: Bar chart with error bars showing mean number of resin tags according to groups and subgroups for APICAL section

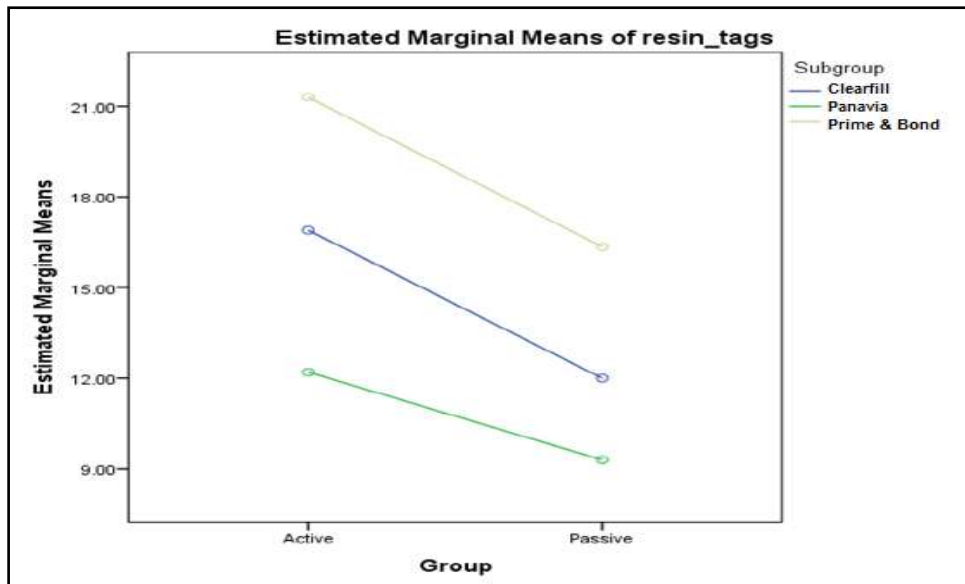


Figure 11: Mean number of resin tags for each group according to subgroups for CORONAL section

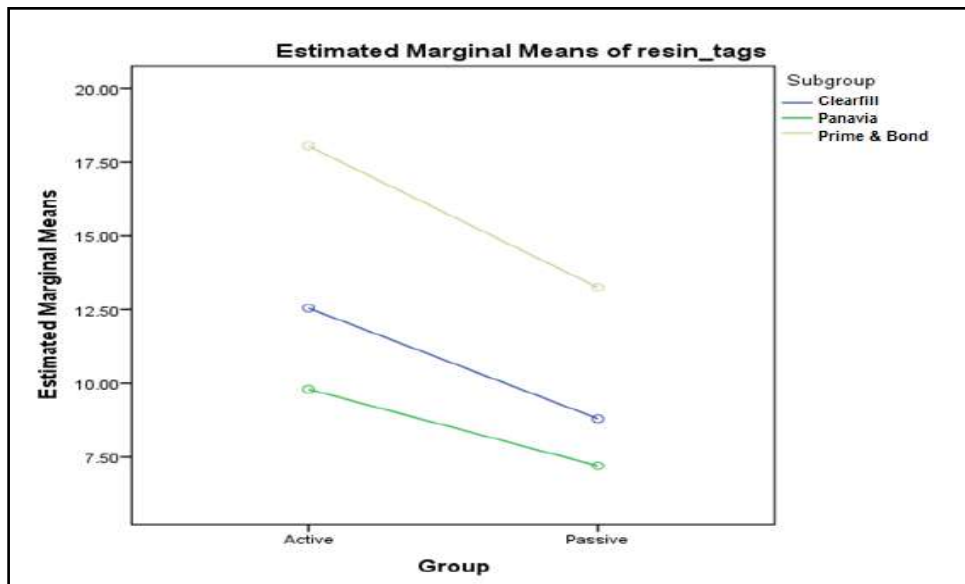


Figure 12: Mean number of resin tags for each group according to subgroups for MIDDLE section

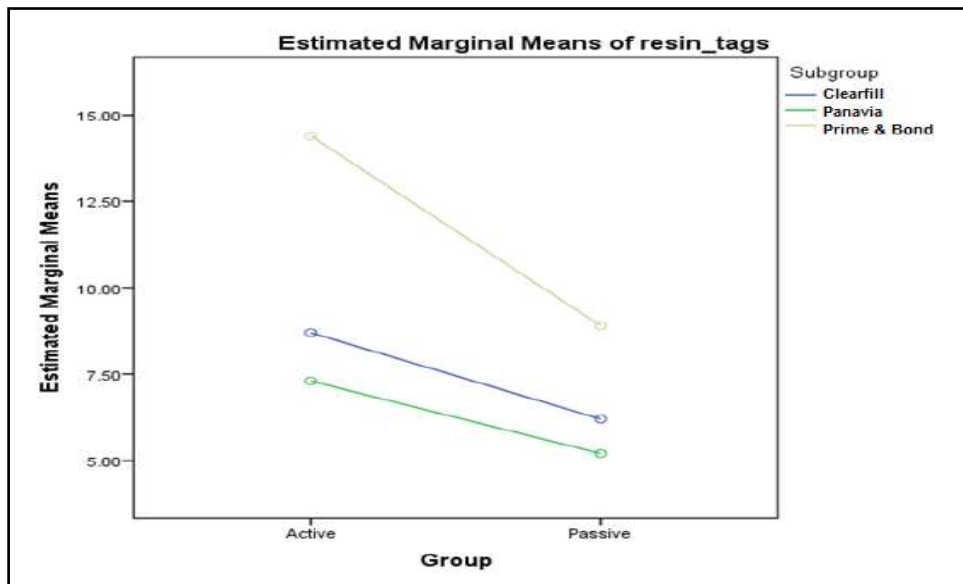


Figure 13: Mean number of resin tags for each group according to groups for APICAL section

ANNEXURE

Group I: ACTIVE IRRIGATION [Sub Group A]

CORONAL			MIDDLE			APICAL		
Sample	Hybrid Layer	Resin Tags	Sample	Hybrid Layer	Resin Tags	Sample	Hybrid Layer	Resin Tags
1	4.71um	17	1	4.60um	13	1	2.36um	8
2	4.56um	19	2	3.90um	14	2	2.67um	10
3	4.07um	18	3	3.14um	15	3	3.02um	7
4	5.06um	15	4	4.97um	10	4	3.80um	11
5	5.16um	17	5	4.11um	14	5	3.12um	9
6	5.89um	20	6	4.78um	13	6	3.45um	11
7	6.00um	19	7	3.47um	12	7	3.21um	6
8	5.92um	16	8	3.89um	15	8	3.59um	6
9	6.05um	18	9	3.65um	12	9	3.42um	7
10	5.87um	15	10	4.40um	11	10	3.39um	8
11	5.15um	14	11	4.15um	11	11	3.60um	8
12	5.20um	16	12	4.99um	15	12	3.78um	6
13	5.67um	19	13	4.83um	12	13	3.97um	10
14	4.98um	17	14	4.05um	13	14	4.26um	11
15	5.16um	18	15	4.69um	14	15	3.49um	11
16	5.32um	16	16	3.86um	9	16	4.18um	8
17	5.56um	15	17	4.53um	10	17	3.16um	9
18	5.63um	16	18	4.47um	13	18	4.04um	10
19	5.38um	16	19	4.41um	12	19	3.93um	9
20	5.89um	17	20	4.76um	13	20	4.21um	9

Group II: PASSIVE IRRIGATION [Sub Group A]

CORONAL			MIDDLE			APICAL		
Sample	Hybrid Layer	Resin Tags	Sample	Hybrid Layer	Resin Tags	Sample	Hybrid Layer	Resin Tags
1	3.55um	14	1	4.99um	10	1	4.09um	5
2	2.98um	10	2	3.11um	9	2	2.14um	10
3	3.00um	11	3	1.42um	11	3	1.86um	8
4	3.14um	9	4	2.10um	8	4	1.34um	6
5	2.13um	13	5	2.09um	9	5	2.05um	11
6	2.44um	15	6	2.26um	11	6	1.63um	5
7	3.50um	10	7	2.00um	10	7	1.39um	8
8	3.36um	11	8	1.90um	8	8	1.74um	4
9	3.67um	16	9	2.04um	8	9	3.52um	7
10	3.00um	13	10	1.32um	7	10	2.04um	5
11	3.21um	14	11	1.47um	9	11	1.38um	6
12	2.85um	15	12	2.00um	7	12	1.92um	5
13	4.02um	8	13	2.16um	6	13	2.22um	6
14	2.48um	10	14	3.67um	7	14	2.06um	5
15	3.60um	12	15	1.63um	11	15	1.55um	7
16	3.70um	13	16	3.06um	9	16	1.90um	6
17	2.69um	14	17	1.83um	12	17	2.12um	4
18	4.34um	9	18	1.38um	7	18	1.35um	5
19	3.04um	12	19	1.95um	10	19	2.52um	7
20	3.26um	11	20	3.10um	7	20	1.76um	4

Group I: ACTIVE IRRIGATION [Sub Group B]

CORONAL			MIDDLE			APICAL		
Sample	Hybrid Layer	Resin Tags	Sample	Hybrid Layer	Resin Tags	Sample	Hybrid Layer	Resin Tags
1	2.88um	11	1	2.54um	11	1	3.34um	5
2	3.40um	13	2	2.96um	13	2	2.23um	7
3	3.99um	16	3	3.12um	9	3	2.54um	6
4	4.88um	9	4	4.69um	12	4	3.80um	5
5	4.89um	10	5	5.21um	8	5	3.46um	10
6	3.45um	13	6	2.78um	10	6	3.67um	8
7	3.96um	11	7	3.67um	9	7	3.08um	7
8	3.53um	12	8	3.78um	7	8	3.29um	11
9	4.17um	10	9	4.63um	8	9	3.26um	6
10	3.35um	14	10	5.10um	11	10	4.05um	5
11	4.20um	11	11	4.31um	10	11	3.48um	9
12	5.01um	10	12	2.88um	9	12	3.65um	7
13	3.49um	13	13	4.45um	12	13	3.84um	8
14	4.02um	9	14	3.94um	13	14	4.01um	7
15	4.92um	12	15	5.01um	11	15	4.23um	11
16	4.87um	15	16	4.15um	7	16	3.04um	5
17	4.82um	17	17	4.86um	10	17	3.26um	10
18	5.24um	11	18	4.19um	9	18	3.11um	6
19	3.95um	13	19	4.22um	9	19	2.98um	5
20	4.73um	14	20	3.99um	8	20	3.68um	8

Group II: PASSIVE IRRIGATION [Sub Group B]

CORONAL			MIDDLE			APICAL		
Sample	Hybrid Layer	Resin Tags	Sample	Hybrid Layer	Resin Tags	Sample	Hybrid Layer	Resin Tags
1	3.03um	8	1	3.76um	5	1	3.15um	5
2	3.15um	7	2	3.11um	8	2	2.10um	7
3	2.50um	9	3	2.50um	6	3	2.03um	4
4	2.20um	10	4	2.09um	10	4	2.11um	5
5	2.12um	7	5	2.16um	5	5	2.21um	4
6	2.16um	10	6	1.50um	11	6	1.36um	5
7	1.90um	12	7	2.18um	7	7	1.47um	6
8	1.60um	11	8	3.01um	8	8	2.14um	4
9	2.08um	9	9	2.00um	6	9	1.77um	5
10	1.35um	10	10	2.29um	9	10	1.19um	6
11	1.87um	11	11	1.36um	5	11	1.35um	5
12	2.08um	6	12	1.35um	6	12	3.00um	4
13	2.26um	8	13	1.75um	11	13	1.63um	5
14	3.01um	9	14	2.12um	7	14	1.29um	6
15	2.10um	11	15	1.67um	8	15	2.10um	5
16	1.75um	9	16	1.56um	10	16	1.15um	4
17	2.29um	6	17	1.63um	5	17	1.86um	6
18	1.83um	12	18	1.55um	6	18	1.22um	5
19	1.96um	10	19	1.77um	5	19	1.34um	8
20	2.22um	11	20	1.47um	6	20	1.53um	5

Group I: ACTIVE IRRIGATION [Sub Group C]

CORONAL			MIDDLE			APICAL		
Sample	Hybrid Layer	Resin Tags	Sample	Hybrid Layer	Resin Tags	Sample	Hybrid Layer	Resin Tags
1	8.50um	25	1	5.22um	15	1	6.11um	15
2	6.90um	23	2	6.77um	17	2	5.30um	17
3	7.16um	21	3	6.57um	19	3	5.63um	16
4	5.19um	19	4	6.90um	17	4	5.85um	14
5	6.47um	24	5	6.49um	21	5	5.21um	12
6	5.15um	18	6	5.94um	19	6	6.23um	14
7	6.78um	17	7	5.67um	16	7	5.76um	16
8	6.87um	21	8	6.98um	19	8	6.23um	12
9	6.23um	25	9	6.04um	17	9	6.04um	13
10	6.26um	22	10	6.61um	23	10	5.71um	13
11	6.93um	21	11	6.44um	17	11	5.50um	17
12	5.91um	20	12	5.94um	16	12	5.61um	16
13	7.21um	21	13	5.83um	18	13	5.31um	15
14	6.05um	23	14	6.47um	17	14	6.39um	14
15	7.23um	19	15	7.11um	18	15	5.44um	13
16	6.12um	20	16	7.51um	21	16	6.90um	11
17	6.60um	25	17	5.71um	18	17	5.13um	16
18	6.31um	25	18	5.95um	17	18	6.23um	17
19	5.89um	18	19	6.39um	20	19	6.87um	14
20	5.54um	19	20	6.01um	16	20	5.89um	13

Group II: PASSIVE IRRIGATION [Sub Group C]

CORONAL			MIDDLE			APICAL		
Sample	Hybrid Layer	Resin Tags	Sample	Hybrid Layer	Resin Tags	Sample	Hybrid Layer	Resin Tags
1	4.59um	19	1	4.22um	9	1	4.44um	10
2	5.44um	16	2	3.62um	11	2	3.36um	11
3	5.96um	15	3	3.79um	14	3	5.11um	9
4	5.50um	14	4	5.10um	12	4	3.89um	5
5	5.48um	18	5	5.23um	11	5	4.62um	7
6	4.95um	15	6	4.67um	16	6	5.74um	8
7	4.00um	16	7	5.16um	14	7	3.49um	6
8	6.12um	20	8	5.31um	10	8	4.13um	13
9	5.13um	17	9	4.56um	11	9	5.16um	9
10	5.68um	13	10	4.99um	18	10	3.44um	11
11	5.35um	21	11	3.87um	17	11	3.79um	10
12	5.26um	16	12	5.53um	19	12	5.98um	9
13	5.91um	16	13	5.83um	15	13	4.98um	8
14	5.78um	15	14	5.76um	9	14	2.99um	12
15	5.88um	19	15	5.80um	13	15	3.09um	6
16	4.67um	14	16	3.49um	11	16	4.27um	7
17	5.19um	18	17	5.27um	10	17	4.57um	10
18	5.21um	15	18	4.35um	14	18	4.71um	6
19	4.99um	17	19	5.96um	16	19	3.21um	12
20	5.99um	13	20	3.09um	15	20	3.02um	9