COMPARATIVE EVALUATION OF QUALITY OF HYBRID LAYER FORMATION AND MICROTENSILE BOND STRENGTH IN SELF- ETCH VS ETCH-AND-RINSE STRATEGIES AFTER USE OF TWO UNIVERSAL ADHESIVES WITH VARYING EVAPORATION PROTOCOL: A CONFOCAL STUDY

Dissertation submitted to

Maharashtra University of Health Sciences, Nashik

in the Partial Fulfillment of Regulations

for the award of the Degree of

MDS

IN

CONSERVATIVE DENTISTRY AND ENDODONTICS

BRANCH – IV

2021

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LIST OF ABBREVIATIONS

Sr. No.	Abbreviation	Full Form
01.	SEM	Scanning Electron Microscope
02.	TEM	Transmission Electron Microscope
03.	CLSM	Confocal Laser Scanning Microscope
04.	RDI	Resin Dentin Interface
05.	Min	Minutes
06.	s	Seconds
07.	RITC	Rhodamine B IsoThioCynate
08.	HL	Hybrid Layer
09.	RT	Resin Tags
10.	OSHA	Occupational Safety and Health Administration
11.	CDC	Centre for Disease Control
12.	LED	Light Emitting Diode
13.	SPSS	Statistical Package for the Social Sciences
14.	µm	Micrometer
15.	mm	Millimeter
16.	HSD	Honest Significant Difference
17.	SD	Standard Deviation
18.	S	Significant
19.	NS	Not Significant
20.	HS	Highly Significant
21.	N	Number of specimens
22.	n	Number of specimens in each group
23.	p-value	Probability of obtaining a test statistic at least as extreme as the one that was actually observed

24.	Max.	Maximum
25.	Min.	Minimum
26.	CI	Confidence Interval
27.	No.	Number
28.	mW/cm2	Milliwatt/square centimeter
29.	UTM	Universal Testing Machine
30.	Bis-GMA	Bisphenol A Glycidyl Methacrylate
31.	HEMA	2-hydroxyethyl methacrylate
32.	MDP	10-Methacryloyloxydecyl dihydrogen phosphate
33.	UDMA	Urethane dimethacrylate

INTRODUCTION

"The real voyage of discovery consists not in seeking new landscapes
but in having new eyes."

-Marcel Proust

Dental adhesives are crucial determinants of effective sealing and retention of contemporary resin-based composite restorations, which have gained increased popularity amongst dentists globally. Adhesion is essentially a complex phenomenon which involves physical and chemical mechanisms that allow attachment of one material to another.

Adhesion to enamel has remained consistently simple and reliable since the introduction of the acid-etch technique in **1955 by Michael Buonocore.**[4]

However, the quest for establishing durable adhesion to dentin has been an arduous task since several pioneer researchers **(Kramer et al. 1952**[1]**, Brudevold et**

al. 1956[2]**, McLean 1996**[3]**)** used glycerol phosphoric acid dimethacrylate, a phosphate monomer, to achieve bonding with dentine.

The bonding principle of dental adhesives is based on the formation of a hybrid layer **(Nakabayashi et al. 1991**[5]**)** as well as the penetration of adhesive into dentinal tubules and the formation of 'resin tags' **(Titley et al. 1995** [6]**, Ferrari & Davidson 1996**[7]**).**

Van Meerbeek, et al. proposed a simple classification of bonding agents based on the interaction of adhesives with dental substrates and number of steps: etch-and-rinse (two- and three-step adhesives), self-etch (one- and two-step adhesives). While Etch & rinse technique involves the use of 30-40% phosphoric acid etchant to remove the smear layer, the self-etch approach is based on the use of non-rinse acidic monomers that simultaneously condition and prime dentin and enamel.[8]

Adhesive systems have progressed from the largely ineffective generations of the 1950s and early 1970s **(Buonocore et al. 1956**[10]**, Bowen et al. 1965**[11]**)** to the relatively successful total- and self-etching systems available contemporarily.

The seventh-generation bonding systems were introduced in late 1999 and early 2005, which represents the latest simplification of adhesive systems. These systems enable the constituents required for bonding to be placed in and delivered from a single bottle. More recently, a new generation of bonding agents has been introduced as the universal or multi-mode adhesives[9], which may be used either as etch-and-rinse or as self-etch adhesives.

Adhesive procedures require smear layer treatment to increase the interaction with the dental substrate. Whereas the smear layer is completely removed by acid etching with etch-and-rinse adhesives, self-etching adhesives need a different approach, based on the pH of the primer or bonding agents. On the other hand, universal adhesives are similar to single-bottle one-step self-etch adhesive systems and can be used with either of the techniques.

Adhesion being a technique-sensitive phenomenon necessitated dental adhesives to witness substantial transformations in their chemical compositions and number of components within the past few decades to achieve durable bonding to dentin using resin monomers. Since the intrinsic wetness of the dentin substrate is a reality[8], hydrophilic monomers have been incorporated into the composition of dentin bonding system for years.

Dissolution of monomers in a solvent enhances extent of diffusion in the porous conditioned substrate, especially in dentin due to its hydrophilic nature. In current adhesives, water, ethanol, and acetone are the commonly used solvents.[12]

Solvents are critical in ensuring diffusion of monomers into the humid demineralized dentin substrate and represent an important role in removing moisture from the substrate during the evaporation. However, the solvents must be eliminated from adhesive after diffusion[14], otherwise remaining solvent may jeopardize polymerization due to the dilution of monomers and may result in voids and increase the permeability of the adhesive layer.[15]

Dickens et al. (2005)[19] reported that when the solvent's concentration is too high, reduced photoinitiator concentration and occupation of voids between the monomer molecules causes reduction in the conversion of monomers to polymers and resultantly reduces the mechanical properties and strength of the bonding. Therefore, after bonding resin applications, the solvent is removed from the resin using airstream.

Spreafico et al. (2006) observed that the use of air-jet after the application of adhesive improves the evaporation of solvent and water, eventually reducing the thickness of adhesive layer and formation of a uniform layer of adhesive on the substrate.[20]

However, variations in the duration of solvent evaporation can occur routinely in clinical practice. Present literature search elucidated that various researchers have studied the effect of air-evaporation time on dentin bond strength of adhesives. Most of the studies demonstrated that increasing the solvent evaporation time results in higher bond strengths values **(Jacobsen et al., 2006; Sadr et al., 2007; Furuse et al., 2008; Ikeda et al., 2008).**[21,15,22,16]

Recently, **Ikeda et al. (2008)**[16] reported that air-drying of Self-Etch Adhesives (SEA) has a significant effect on the mechanical properties of the adhesive upon setting. They also concluded that it is beneficial to remove solvents of the SEAs as much as possible by thorough, strong air-drying in order to achieve a strong adhesive layer at the interface.

Chiba et al. (2006) examined the effect of air-drying time of adhesives on the dentin bond strength of several single application self-etch adhesive systems and observed that when the adhesives were not air dried, small pores and cracks, which could lead to catastrophic failure of the bonding, were observed inside the cured adhesives. Moreover, they also concluded that prolonged drying was very detrimental to bond strength.[23]

Thus, contemporarily there is no consensus regarding the appropriate solvent evaporation duration for universal adhesives to achieve predictable bonding. Also, the effects of extended solvent evaporation of these adhesive systems upon quality of hybrid layer formation and its inter-relation to microtensile bond strength have not been vastly studied.

Understanding of an interfacial phenomenon such as adhesion has direct relevance and practical benefit in revolutionizing and extending usage of dental adhesives. The assessment of bonding involves both qualitative and quantitative aspects.

Microscopic techniques, especially confocal laser scanning microscope (CLSM) offers several advantages in investigating qualitative aspect of bonding by its 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.[17]

Ever since the introduction of Microtensile bond strength (μTBS) test in 1994, researchers have relied on its contribution to adhesion testing immensely due to its greater discriminative capability than the traditional macro-shear bond test.[18]

Salvio LA et al. 2013 evaluated the hybridization quality and bond strength of SEA and Etch-and-rinse adhesives to dentin and concluded that the adhesive systems demonstrated particular hybridization quality and bond strength, which depended upon not only the actual category of the adhesives, but also on the composition of the adhesives and the interaction to dentin. The hybridization quality is related with the immediate microtensile bond strength to dentin and is essential to improve the bonding effectiveness.[24]

However, the relationship between quality of hybrid layer formation and microtensile bond strength of universal adhesives in various bonding strategies has not been vastly studied. Also, it is of paramount importance to explicate and standardize the duration of air evaporation protocols of universal adhesives in both self-etch and etch-and-rinse strategies to achieve predictable bonding of restorations in routine dental practice.

Thus, the aim of this in vitro study was to evaluate and compare the quality of hybrid layer formation and microtensile bond strength of two universal adhesives in self-etch vs etch-and-rinse strategies when used with varying evaporation protocols.

The null hypothesis was that there is no significant difference in the quality of hybrid layer formation and microtensile bond strength in self-etch and etch-and-rinse strategies after use of these universal adhesives with varying evaporation protocol.

AIMS AND OBJECTIVES

Aim

To evaluate and compare the quality of hybrid layer formation and microtensile bond strength in self-etch vs etch-and-rinse strategies after use of two universal adhesives i.e 3M ESPE Single Bond Universal and Ivoclar Vivadent Tetric N-bond Universal with varying evaporation protocol.

Objectives

1. To evaluate quality of hybrid layer formation in self-etch vs etch-and-rinse strategies after use of two universal adhesives i.e 3M ESPE Single Bond Universal and Ivoclar Vivadent Tetric N-bond Universal with varying evaporation protocol as observed under confocal microscope.

2. To compare quality of hybrid layer formation in self-etch vs etch-and-rinse strategies after use of two universal adhesives i.e 3M ESPE Single Bond

Universal and Ivoclar Vivadent Tetric N-bond Universal with varying evaporation protocol as observed under confocal microscope.

3. To evaluate the microtensile bond strength in self-etch vs etch-and-rinse strategies after use of two universal adhesives i.e 3M ESPE Single Bond Universal and Ivoclar Vivadent Tetric N-bond Universal with varying evaporation protocol.

4. To compare the microtensile bond strength in self-etch vs etch-and-rinse strategies after use of two universal adhesives i.e 3M ESPE Single Bond Universal and Ivoclar Vivadent Tetric N-bond Universal with varying evaporation protocol.

5. To analyze the relationship between quality of hybrid layer formation and microtensile bond strength if any.

REVIEW OF LITERATURE

The current strategies for achieving predictable bonding with tooth substrate are based on the evolution of different dental adhesives and techniques combined with the ever-growing expertise and understanding of the clinician. Therefore, it is imperative to understand the mechanisms, principles and strategies of bonding that have evolved over time; and the challenges encountered by researchers and clinicians that are associated with this technique sensitive procedure.

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.

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.

Watson TF et al in **1989** conducted a Confocal Optical Microscope Study of the Morphology of the Tooth/Restoration Interface using Scotchbond 2 Dentin Adhesive and observed that the sections showed excellent adaptation of the adhesive to the tooth surface, with considerable penetration into the etched enamel structure. They further stated that observation of the distribution of the bonding agent at the interface was possible without any fluorescent markers, the clear resin of the adhesive being visualized as transparent or dark band. However, it was not possible for the researchers to visualize the distribution of the air-inhibited layer within the overlying composite restoration in this study.

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 filing 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.

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.

Miyazaki et al in **1996** conducted a study to determine the influence of dentin primer application methods on bond strength to human dentin. They investigated the influence of dentin primer application procedures and drying time of the primed dentin surface on bond strength to dentin. They concluded that the retained organic solvent and water within the adhesive resin can severely compromise the structural integrity of the hybrid layer. The polymerization reaction of the bonding agents might be inhibited by the presence of primers if inadequate drying occurs. Desiccation of the primed dentin surface may alter the nature of the hybrid layer. Both of these would tend to reduce the bond strength.

Gwinnet et al in **1996** determined the quantitative contribution of dentin hybridization to bonded assembly strength and demonstrated the micromorphology of the interface with and without collagen present. They stated that hybrid layer is essential for formation of bond between dentin and adhesive materials. They corelated the mechanism of primary attachment with the zone of partially demineralized dentin.

According to them, the etched and totally demineralized dentin which consists of collagen network only seems to be of minor importance. They further stated that reinforcement of the acid-induced porosities in the partially demineralized dentin by the monomers of the bonding agent is a pre-requisite for the stability of this zone and the remaining porosities may have a negative impact on the bond.

Kimura et al in **1997** studied the visualization and quantification of dentin structure using confocal laser scanning microscopy (CLSM) and stated that confocal microscopy is well suited to the observation of dental and material surfaces and to monitoring the effects of various agents and factors on their microstructure. They further stated that surface images obtained using CLSM were similar to those observed by scanning electron microscopy (SEM) and eliminated the need for sample-altering conventional SEM preparation techniques. This fluorescence technique offers a useful new alternative for visualization and quantification of dentin.

Marshall et al in 1997 studied the comparative effects of dentin depth and pH of acid on demineralization of dentin to determine if the dentin demineralization rates are proportional to acid concentration for demineralization in phosphoric acid. In this study, depth changes with respect to the reference layer were determined for the intertubular and peritubular dentin to quantify structural changes. It was concluded that peritubular dentin etching rates increased with decreasing pH, but changes were not linear. Also, intertubular dentin surface recession was small and plateaued for low concentrations. The peritubular etching rate and intertubular dentin recession did not depend on dentin depth.

Armstrong SR et al in **1998** investigated the tensile bond strength of two dental adhesives using the recently introduced microtensile bond strength testing design and verified the failure mode for each test specimen with scanning electron microscopy. They concluded that the microtensile test methodology shows promise, due to its versatility and significant potential for in vivo applicability, as a standardized strength-based test methodology for adhesive dental restorative systems.

Paul et al in **1999** evaluated the influence of increasing amounts of water on the physical properties of a model dentine primer resin (HEMA) and model dentine bonding resin (a mixture of HEMA and Bis-GMA). They concluded that Higher water content lowered the physical properties. The plasticizing effects of extrinsic water are far more important than the effects of intrinsic water in poly-HEMA resin. Hybrid layers composed primarily of poly-HEMA would be expected to be more elastic than those made with bifunctional, cross-linked polymer chains.

Gallo et al in **2000** measured and compared the shear bond strength to dentin when four fifth-generation dentin bonding agents were applied immediately after dispensing or allowed to evaporate for 10 minutes prior to application. Two of the four bonding agents contained acetone (Prime & Bond 2.1, BISCO One Step), one contained ethanol (3M Single Bond) and one contained water (One Coat Bond). They found that a 10-minute delayed application of acetone, ethanol and water-containing fifth generation dentin bonding agents did not produce a statistical decrease in bond strengths compared to bonding agents used immediately after dispensing. However, the acetone-containing bonding agents demonstrated a trend toward decreased bond strengths when the bonding agents were dispensed 10 minutes prior to their application.

Reis et al in **2003** conducted a study to evaluate the microtensile bond strength (MTBS) of ethanol/water- and acetone-based, one-bottle adhesive systems to enamel and dentin in the presence or absence of their respective solvents. They found that there were no statistically significant differences in mean bond strength among the groups restored with or without solvent for enamel. However, the results were significantly different for the dentin groups. SEM examination indicated that the dentin group failure modes were significantly different from the enamel groups. The results suggest that the presence of organic solvents does not influence TBS to enamel. However, MTBS to dentin was significantly affected by the absence of solvents in the adhesive system.

Cho et al in 2004 investigated the hypothesis that varying the acetone content of single solution dentin bonding agents may affect the adhesive layer thickness and microtensile bond strength (MTBS) of the bonded complex, and explored whether the adhesive layer thickness is a valid predictor for MTBS.

Experimental dentin bonding agents containing (27, 37, 47, 57, or 67) mass fraction% acetone were used to bond composite resin onto occlusal dentin surfaces of extracted human molars. It was concluded that the acetone content affected the adhesive layer thickness and the MTBS of the bonded complex. However, the hypothesis was partly rejected because the adhesive layer thickness failed to predict the MTBS. The increased crack probability due to evaporation of acetone, poor polymerization and low strain capacity of the resulting adhesive layer was suggested as a mechanism for lower MTBS when using bonding agents with high amounts of acetone. Lower acetone concentrations did not seem to lower MTBS, but rather improved the integrity of dentin/adhesive bond.

Yiu et al in **2005** examined the extent of organic solvent and water retention in comonomer blends with different hydrophilicity after solvent evaporation, and the extent of tracer penetration in polymerized films prepared from these resins. They concluded that

(1) The percentage of solvent retained in acetone-based and ethanol-based adhesive mixtures increased significantly with increasing hydrophilicity of the neat, non-solvated resin blends.

(2) More organic solvent and water were retained in the ethanol-based adhesive mixtures when compared with acetone-based adhesive after evaporation.

(3) The addition of water to comonomer–solvent mixtures allow water to hydrogen bond with comonomers, resulting in a large increase in retention of water in these solvated adhesive mixtures.

Chiba et al in **2006** examined the effect of air-drying time of adhesives on the dentin bond strength of several single application self-etch adhesive systems. The adhesive/resin composite combinations used were: Adper Prompt L-Pop/Filtek Z250 (AP), Clearfil Tri-S Bond/Clearfil AP-X (CT), Fluoro Bond Shake One/Beautifil (FB), G-Bond/Gradia Direct (GB) and One-Up Bond F Plus/Palfique Estelite (OF). They observed that with longer air drying of adhesives, no significant changes in bond strengths were found for the systems used except for OF. Significantly lower bond strengths were obtained for the 10-second air-drying group for OF. The data suggested that, with four of the single application self-etch adhesive systems, air drying is essential to obtain adequate dentin bond strengths but increased drying time does not significantly influence bond strength. For the other system studied, the bond strength of the non-air-dried group was not significantly different from the five second drying time, but prolonged drying was very detrimental to bond strength. For all five of the systems studied, a five-second air-drying time appeared to be appropriate.

Sadr et al in **2007** investigated the performance of two all-in-one adhesives i.e Clearfil Tri-S bond (TS) and Clearfil SE bond (SE) and whether it was affected by air drying of the solvent containing agent. Micro-shear bond strengths to human dentin after solvent air-drying times of 2, 5 or 10 s for each group were measured (n = 10). The indentation creep and hardness of the bonding layer were also determined for each group. The lowest micro-shear bond strength, nano-indentation hardness and creep stress exponents were obtained for 2 s air dried specimens of each material. After 10 s air blowing, SE showed superior properties compared to TS groups. The results of the micro-shear bond strength test indicated that when properly handled, SE bonded more effectively to dentin than TS, and that the air-drying time of the self-etching material can significantly affect the bond strength. They concluded that air-drying is a crucial step in the application of solvent containing adhesives and may affect the overall clinical performance of them, through changes in the bond strength and altering nano-scale mechanical properties.

Bhuvaneswaran et al in **2007** investigated the resin-dentin interface using acetone- based, water-based, and water-and-alcohol based single component dentin bonding agents in dry and moist conditions under a confocal laser scanning microscope (CLSM). They concluded that:

1. The maximum width of hybrid layer formation and maximum length of resin tags was best achieved when the dentin was kept moist by blotting and when acetone-based adhesive was used.
2. Maximum infiltration is seen when the dentin is kept moist after acid etching.
3. The best way of keeping the acid-etched dentin moist is by blotting the excessive moisture using absorbent paper.
4. Acetone-based adhesive was able to provide better hybridization compared to the water-based and water-and-ethanol based bonding agents.

Ikeda et al in 2008 studied the effect of air-drying and solvent evaporation on the strength of HEMA-rich versus HEMA-free one-step adhesives. The objectives of this study were

1. To clarify the relationship between the duration of air-drying of one-step self-etch adhesives (1-SEAs) and the evaporation degree (ED) of solvents

2. To evaluate the effect of ED on the ultimate micro-tensile strength (μTS) of the adhesives.

It was found that a longer air-drying time for 10-s resulted in a statistically significantly higher μTS for the HEMA-rich Clearfil S3 Bond. The μTS of the latter was higher than that of the other two HEMA-free adhesives for each air-drying time. They concluded that air-drying of 1-SEAs had a significant effect on the degree of solvent evaporation (ED) and also on the mechanical properties (µTS) of the 1-SEAs upon setting. It is therefore beneficial to remove solvents of the 1-SEAs as much as possible by thorough, strong air-drying in order to achieve a strong adhesive layer at the interface.

Klein-Junior et al in **2008** evaluated the effect of a warm or cold air-dry stream for solvent evaporation on the microtensile resin–dentin bond strength (µTBS), nanoleakage pattern (SEM), degree of conversion (DC) and solvent evaporation rates (SE) of an ethanol/water- (Adper Single Bond, 3MESPE) and an acetone-based (Prime & Bond 2.1, Dentsply), two-step etch-and-rinse adhesive system. Higher µTBS and lower nanoleakage were observed when the SE step was performed with warm air-dry stream. However, the DC of the adhesives was not altered by the use of a warm air-dry. They concluded from the study that the use of a warm air-dry stream seems to be a clinical tool to improve the bond strength and the quality of the hybrid layer (less nanoleakage infiltration), since it might reduce the number of pores within the adhesive layer.

Cadenaro et al in **2009** examined the extent of ethanol retention in five comonomer blends of experimental methacrylate-based dental adhesives, containing (10, 20, or 30 wt.%) ethanol, after solvent evaporation, as well as observing the effect of residual ethanol and exposure duration on degree of conversion (DC). They observed that the concentration of retained ethanol increased significantly with ethanol concentration ($p < 0.01$). As ethanol is evaporated from solvated comonomer mixtures, the molar concentration of comonomers increases, reducing the vapor pressure of the remaining ethanol. Thus, the fractional loss of ethanol solvent decreases as the comonomer concentration increases. In their study, they concluded that even with prolonged evaporation, 4–9% residual ethanol concentration can remain in 90/10 (wt./wt.) comonomer–ethanol mixtures. This is thought to be because comonomers lower the vapor pressure of ethanol. This amount of residual ethanol facilitates DC but lowers the rate of polymerization.

Sauro et al in **2012** conducted a study to assess the quality of the resin–dentin interfaces created with selected bonding parameters by using confocal microscopy (CLSM), AFM nano-indentation and microtensile bond strength test (TBS). Dentin conditioned with phosphoric acid or EDTA was bonded in ethanol- or water-wet condition using a HEMA-free or HEMA-containing adhesive. The resin-bonded teeth were stored in distilled water (24h) and sectioned as matchsticks (0.9mm2) for TBS. Further resin bonded teeth were sectioned and analyzed using CLSM, and AFM nano-indentation. It was found that the HEMA-containing adhesive applied onto phosphoric acid-etched ethanol or water-wet dentin created hybrid layers with the lowest biomechanical nano-properties ($p < 0.05$); no significant differences in TBS were found between the two wet-bonding techniques ($p > 0.05$). However, the ethanol-wet bonding reduced the dye penetration into the adhesive layer created with the HEMA-containing adhesive. Hybrid layers with high biomechanical properties, low micropermeability and no shrinkage were only possible when using HEMA-free adhesive applied in ethanol wet-dentin. The use of HEMA-free adhesives applied onto ethanol-wet dentin may be considered as an alternative and suitable bonding strategy to achieve high quality resin–dentin interfaces.

Wagner et al in **2014** conducted a study to compare the microtensile bond strength (mTBS) and resin penetration into dentine of three universal adhesives (UAs) applied in two different two etching modes i.e., self-etch or etch-and-rinse. The bonding systems evaluated were Futurabond Universal, Scotchbond Universal Adhesive and All-Bond Universal. They observed that the addition of an etching step did not significantly affect the mTBS of none of the UAs, when compared to their self-etch application mode. All pre-etched specimens showed considerably longer resin tags and thicker hybrid layers. Thus, they concluded that application of an etching step prior to UAs improves their dentine penetration but does not affect their bond strength to dentine after 24 h or after thermocycling. Similar bond strength values were observed for the UAs regardless of the two application modes, which makes them reliable for working under different clinical conditions.

Perdiago et al in **2014** evaluated the 18-month clinical performance of a multimode adhesive (Scotchbond Universal Adhesive, SU, 3M ESPE, St Paul, MN, USA) in non-carious cervical lesions. The NCCLs received the SU adhesive system applied in different modes: an etch-and-rinse approach, keeping the dentin moist (ERm) or dry (ERd), and a self-etch approach with (Set) or without (SE) selective enamel etching. At 18 months, there were no differences in postoperative sensitivity between any pair of groups in this study. It was concluded from the results of this study that the clinical retention of the multimode adhesive at 18 months did not depend on the bonding strategy. The only differences between strategies were found for the parameter marginal adaptation, for which the FDI criteria were more sensitive than the USPHS criteria.

Ramya et al in **2015** evaluated the resin-dentin interface using a self-etch and a total etch adhesive system bonded to moist dentin using confocal laser scanning microscope (CLSM). They observed that the resin dentin interface of conventional total-etch adhesives performed better than newer self-etch adhesive system in terms of resin tag length and hybrid layer thickness. The possible explanations for the better performance by total etch in terms of resin tag length were as follows:

(1) Demineralising action of the strong etchant (phosphoric acid) used in total etch group and subsequent rinsing of the reaction products

(2) The moist bonding technique prevents the collagen fibers from collapsing

(3) The permeability of the demineralized dentin matrix (i.e., maintenance of collagen fibril separation) and the diffusivity of the comonomer mixtures used to infiltrate the demineralized dentin.

Irmak et al in **2016** determined the shear bond strengths of three total-etch adhesives with different solvents (acetone, ethanol or tertiary butanol) applied to air or blot dried moist dentin. They concluded that tertiary butanol-based adhesive showed higher bond strength values than ethanol or acetone-based adhesives. Blot drying of dentin improved the bond strength values of tertiary butanol-based adhesive.

Ahmed et al in **2018**[52] determined the dentin bonding ability of three new universal adhesive systems under different etching modes using microshear bond strength (μSBS). Futurabond U, Single Bond Universal, and Tetric N-Bond Universal were used in this study. wo etching modes (total-etch and self-etch) were employed for each adhesive group. The adhesives were applied on dentin surfaces according to the manufacturer's instructions then composite resin (Z350 XT, nanocomposite) was condensed through a polyethylene tube with a 1 mm internal diameter and 2 mm height attached firmly to dentin surfaces and light cured. The μSBS was measured by using universal testing machine at crosshead speed of 0.5 mm/min. Among the universal adhesives, Futurabond U and Tetric N-Bond Universal in total-etch mode showed significantly higher μSBS values than in self-etch mode. Single Bond Universal did not show any significant difference in μSBS between the total-etch mode and self-etch mode. They concluded that performance of universal adhesives was shown to be material dependent. The results indicated that universal adhesives used on dentine performed better in total-etch mode than self-etch mode.

Yamauchi et al in **2019** investigated the dentin bond fatigue resistance and interfacial science characteristics of universal adhesives through etch-and-rinse and self-etch modes. Resin composite was bonded to human dentin with four universal adhesives, namely, Adhese Universal, All-Bond Universal, G-Premio Bond, and Scotchbond Universal Adhesive. The initial bond strengths, bond fatigue strengths, and interfacial science characteristics of the universal adhesives with dentin through etch-and-rinse and self-etch modes were determined. Bond fatigue resistance (initial bond strength and bond fatigue strength) of universal adhesives in etch-and-rinse mode showed no significant difference in contrast to that in self-etch mode and was material-dependent regardless of the etching mode. Although phosphoric acid conditioning of dentin did not have a strong impact on the bond fatigue resistance, surface free energy and parameters of dentin were significantly decreased by etching and by application of universal adhesives regardless of etching mode.

Cuevas-Suárez et al in **2019** evaluated through a systematic review and meta-analysis whether the immediate and long-term bonding performance of universal adhesives would be improved by prior acid etching. A global analysis comparing self-etch or etch-and-rinse strategies and the influence of aging on bonding performance was performed. It was observed that the enamel bond strength of universal adhesives was improved by the etch-and-rinse approach ($p < 0.05$). In dentin, this effect was observed for ultra-mild and intermediately strong universal adhesives ($p < 0.05$). Irrespective of the strategy employed, intermediately strong adhesives showed a decrease in bond strength after all types of aging. This effect was also observed for ultra-mild universal adhesives used in the etch-and-rinse approach ($p < 0.05$). Mild universal adhesives showed bond strength stability in both strategies ($p > 0.05$). They concluded that in vitro evidence suggests that bonding performance of mild universal adhesives can be improved by using the selective enamel-etch strategy. Mild universal adhesives seem to be the more stable materials, in both etch-and-rinse or self-etch strategies.

Awad et al in **2019** conducted a review to evaluate the effect of air-drying time on the adhesion (bond) strength of adhesives to dentin in previously published studies and a meta-analysis to quantify the differences in the bond strength obtained after the different air-drying times. The included studies were laboratory studies that investigated the effect of adhesive air-drying time on adhesion (bond) strength of resin-based adhesives to coronal dentin. They concluded from their review that the air-drying time of adhesives is crucial to the adhesion strength to coronal dentin. Adhesive air-drying for shorter durations (5–10 s) may be insufficient to obtain adequately durable bonding to dentin, instead, Air-drying should be performed for longer durations (15–30 s), considering the pressure and distance of air-drying source.

Chen et al in **2020** evaluated the air-blowing temperature and water storage time on the micro-tensile bond strength (μTBS) of five universal adhesive systems to dentin. The bond strength with two different air-blowing temperatures (60±2ºC and 23±2ºC) was measured after water storage at 37ºC for 24 h and 100 days respectively. hree-way ANOVA revealed a significant effect of universal system ($p<0.001$) and air-blowing temperature ($p<0.001$) on bond strength to dentin except water-storage time ($p=0.145$). The interaction within three factors was significantly different. It could be concluded that the μTBS of universal systems to dentin was material-depended. The higher and more stable bonding performance of universal systems on dentin could be achieved by air-blowing at 60±2ºC temperature. In addition, the quantity of voids in the adhesive layer of acetone-based universal adhesive was significantly reduced by higher temperature.

MATERIAL AND METHODS

Eighty extracted human premolars 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). The selected teeth were stored in phosphate buffer saline solution (Severn, Biotech).

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

SELECTION CRITERIA

INCLUSION CRITERIA:

1. Sound human extracted premolars
2. Teeth extracted either for orthodontic or periodontal purpose
3. Teeth with intact coronal surface

EXCLUSION CRITERIA:

1. Carious teeth
2. Endodontically treated teeth
3. Teeth affected by trauma, fractured teeth
4. Hypocalcified teeth
5. Teeth with developmental anomalies.

ARMAMENTARIUM:

Instruments and Equipment:

- Straight probe
- Explorer
- Pair of Tweezers
- Excavator
- Hand Scaler (Satelec P5 Newtron Worktop Scaler, Satelec Acteon)
- Digital Vernier calliper (WorkZone Hand Tools, Germany)
- Cotton holder
- Waste receiver
- Mixing pad
- Micro Brush
- Mylar strips
- Tofflemire retainer
- Oil-free air-water syringe
- High speed airotor (NSK, Japan)

- Straight hand piece (NSK, Japan)
- Double sided diamond disc (DFS, Germany)
- Composite instruments (GDC)
- Composite finishing & polishing kit (Shofu Dental Corporation)
- LED Light curing gun (Bluephase N MC, Ivoclar Vivadent, Schaan/Liechtenstein)
- Precision cutting saw (IsoMet 5000, Buehler)
- Grinder & polisher (Buehler)
- Confocal Laser Scanning Microscope (ZEISS with LSM Software ZEN 2007)
- Universal testing machine (ACME Engineers, Model no. UNITEST-10)

MATERIALS

- 0.9% normal saline solution (Eurolife healthcare)
- 0.1% Rhodamine B dye (LobaChemie, India)
- Scotchbond Etchant (3M Espe, St. Paul, USA)
- Tetric N-Bond Universal (Ivoclar Vivadent, Schaan/Liechtenstein)
- Single Bond Universal (3M Espe, St. Paul, USA)
- Filtek Z350 XT Universal Restorative (3M Espe, St. Paul, USA)

SAMPLE PREPARATION

The teeth were sectioned 5 mm beneath the cemento-enamel junction using a double-sided diamond disc (DFS, Germany). The occlusal enamel was removed to expose the middle coronal dentin and further wet polishing was done with 600 grit SiC paper (Struers LaboPol-4. Struers, Copenhagen, Denmark) for 60 seconds to standardize the smear layer.

DISTRIBUTION OF STUDY GROUPS

All the samples were randomly divided into two groups according to the bonding strategy used:

1) Group I: Etch-and-rinse

2) Group II: Self-etch

Group	Bonding Strategy	No. Of Samples
GROUP I	Etch-and-rinse	40
GROUP II	Self-etch	40

In **etch-and-rinse** group, the exposed dentin surfaces were etched with 37% phosphoric acid and rinsed with water. The excess water was removed from the dentin surface with absorbent paper.

In the **self-etch** group, no phosphoric acid etching was done. Universal adhesives were directly applied on the exposed dentin surfaces with gentle rubbing motion for 20 s as per the manufacturer's instructions.

Each group was further subdivided into two sub-groups according to the adhesive used:

1) Sub-group I A: Etch-and-rinse using 3M ESPE Single Bond Universal
2) Sub-group I B: Etch-and-rinse using Ivoclar Vivadent Tetric N-bond Universal
3) Sub-group II A: Self-etch using 3M ESPE Single Bond Universal
4) Sub-group II B: Self-etch using Ivoclar Vivadent Tetric N-bond Universal

Group	**Sub-group**	**No. of Samples**
GROUP I Etch-and-rinse	I A. Single Bond Universal	20
	I B. Tetric N-bond Universal	20
GROUP II Self-etch	II A. Single Bond Universal	20
	II B. Tetric N-bond Universal	20

After application of universal adhesives, evaporation of solvent was done with oil-free air-water syringe. The air pressure was adjusted to 1 bar using a pressure regulator, and the air nozzle was held at a distance of 1.5 cm from the dentin surface.

The samples were further divided according to the experimental solvent evaporation times into four sub-categories. Each sub-group contained 20 samples which were further divided into 5 samples each for varying evaporation time protocol after 0 seconds, 5 seconds, 15 seconds, 25 seconds.

Group	Sub-group	Solvent Evaporation time	No. of Samples
GROUP I **Etch-and-rinse**	I A. Single Bond Universal	I Aa. 0 seconds (control)	5
		I Ab. 5 Seconds	5
		I Ac. 15 Seconds	5
		I Ad. 25 Seconds	5
	I B. Tetric N-bond Universal	I Ba. 0 seconds (control)	5
		I Bb. 5 Seconds	5
		I Bc. 15 Seconds	5
		I Bd. 25 Seconds	5
GROUP II **Self-etch**	II A. Single Bond Universal	II Aa. 0 seconds (control)	5
		II Ab. 5 Seconds	5
		II Ac. 15 Seconds	5
		II Ad. 25 Seconds	5
	II B. Tetric N-bond Universal	II Ba. 0 seconds (control)	5
		II Bb. 5 Seconds	5
		II Bc. 15 Seconds	5
		II Bd. 25 Seconds	5

GROUP I A: ETCH-AND-RINSE STRATEGY WITH SINGLE BOND UNIVERSAL

37% phosphoric acid (Scotchbond Etchant, 3M Espe, St. Paul, USA) was applied on the dentin surface for 15 s followed by a 10 s rinse with water. The excess water was removed from the dentin surface with absorbent paper. Single Bond Universal (3M Espe, St. Paul, USA) was labelled with 0.1% Rhodamine B dye

(LobaChemie, India) and then applied to the dentin surface for 20 seconds using a microbrush. Solvent evaporation was done as per the experimental protocol for 0, 5, 15 and 25 seconds respectively for each sub-group and light cured using a Bluephase N MC (Ivoclar Vivadent, Schaan/Liechtenstein) curing light at an irradiance of 800 mW/cm2 for 10 s.

GROUP I B: ETCH-AND-RINSE STRATEGY WITH TETRIC N-BOND UNIVERSAL

Acid etching of dentin surface was done with 37% phosphoric acid (Scotchbond Etchant, 3M Espe, St. Paul, USA) for 15 s followed by a 30 s rinse with water. The excess water was removed from the dentin surface with absorbent paper. Tetric N-Bond Universal (Ivoclar Vivadent, Schaan/Liechtenstein) was labelled with 0.1% Rhodamine B dye (LobaChemie, India) and then applied to the dentin surface for 20 seconds using a microbrush. Solvent evaporation was done as per the experimental protocol for 0, 5, 15 and 25 seconds respectively for each sub-group and light cured using a Bluephase N MC (Ivoclar Vivadent, Schaan/Liechtenstein) curing light at an irradiance of 800 mW/cm2 for 10 s.

GROUP II A: SELF-ETCH STRATEGY WITH SINGLE BOND UNIVERSAL

0.1% Rhodamine B dye (LobaChemie, India) was used to label Single Bond Universal (3M Espe, St. Paul, USA). A single coat of adhesive was applied using a micro brush tip on the dentin surface in a rubbing motion for 20 seconds and agitated. Bluephase N MC (Ivoclar Vivadent, Schaan/Liechtenstein) light at an irradiance of

800 mW/cm2 for 10 s was used to cure the bonding agent after solvent evaporation for 0, 5, 15 and 25 seconds respectively for each sub-group.

GROUP II B: SELF-ETCH STRATEGY WITH TETRIC N-BOND UNIVERSAL

Tetric N-Bond Universal (Ivoclar Vivadent, Schaan/Liechtenstein) was labelled with 0.1% Rhodamine B dye (LobaChemie, India) and then directly applied to the dentin surface for 20 seconds using a microbrush in a single coat. Solvent evaporation was done according to the experimental protocol for 0, 5, 15 and 25 seconds respectively for each sub-group and light curing was achieved with a Bluephase N MC (Ivoclar Vivadent, Schaan/Liechtenstein) curing light at an irradiance of 800 mW/cm2 for 10 s.

FABRICATION OF RESTORATION

Filtek Z350 XT Universal Restorative (3M Espe, St. Paul, USA), a light cured packable composite resin, was incrementally placed on dentin surfaces of all the samples, with each increment being approximately 1 mm in thickness and light cured for 20 s. All the samples were polished with sandpaper discs (Shofu Dental Corporation, California).

SECTIONING OF SAMPLES

All samples were stored in distilled water for 24 hours after completion of the restorative procedure. Each tooth was sectioned longitudinally in a mesio-distal direction across the bonded interface with a microtome precision (Isomet, Beuhler, Germany) to attain two halves of each tooth specimen.

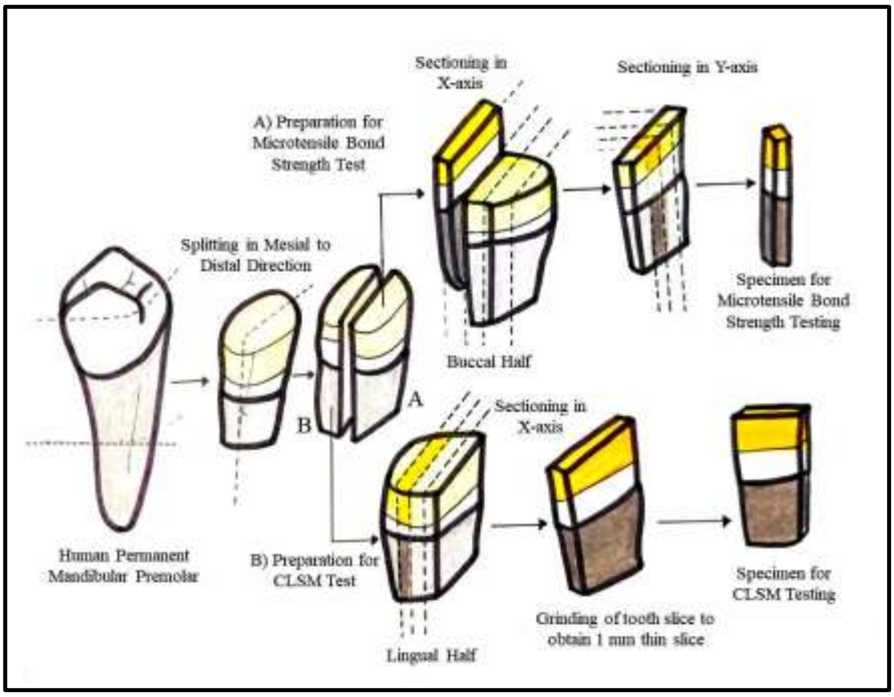


Figure: Sectioning of samples for CLSM and Microtensile Bond strength testing

METHODS OF MEASUREMENTS

Two parameters were measured:

1. Depth of penetration of resin tags into dentin

2. Microtensile bond strength of resin-dentin interface

CONFOCAL MICROSCOPIC EVALUATION

The resulting sections of one half 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 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 425 x 425 µm2, and the resolution was 512 x 512 pixel. From the tooth slicing, five distinct resin tags in each tooth which were continuously extending from the resins-tooth interface and showing the deepest penetration depth in the same frame were taken into account and measured.

In order to elaborate the quality of the hybrid layer, the measurements were performed using CLSM quantification tool on each image, and a mean was calculated. Thus, only one value (mean) per section entered the statistical analysis.

MICROTENSILE BOND STRENGTH TEST

The second half of each specimen was subjected to further longitudinal sectioning in both X and Y directions across the bonded interface to obtain 12mm long sticks/beams with cross-sectional area of 1 mm^2.

Figure: Cutting of the specimen along X and Y axis and the resulting stick

Each beam was attached to testing apparatus of microtensile bond strength testing machine (ACME Engineers, Model no. UNITEST-10) using cyanoacrylate adhesive (Loctite 406 Bonder Cyanoacrylate Instant Adhesive) and tested at a cross-head speed of 0.5mm/min until failure. At this point the load at failure in Kilograms was recorded, and the specimen's fragments cautiously removed from the grips with a scalpel.

The cross-sectional area at the site of fracture was measured to the nearest 0.01 mm with a digital caliper, to calculate the bond strength at failure in Mega Pascals (MPa). The microtensile bond strength values (MPa) were calculated by dividing the load at failure by the cross-sectional bonding area. Final values were express in MPa obtained from the following equation:

$$\mathbf{\mu TBS = F/A} \times \mathbf{0.098}$$

In which, μTBS stands for microtensile bond strength value (MPa), F for microtensile force applied for the test (kgf) and A is the sample bonded area $(mm^2)/100=(cm^2)$.

μTBS results were employed as the average of the tested beams for each adhesive system tested. 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

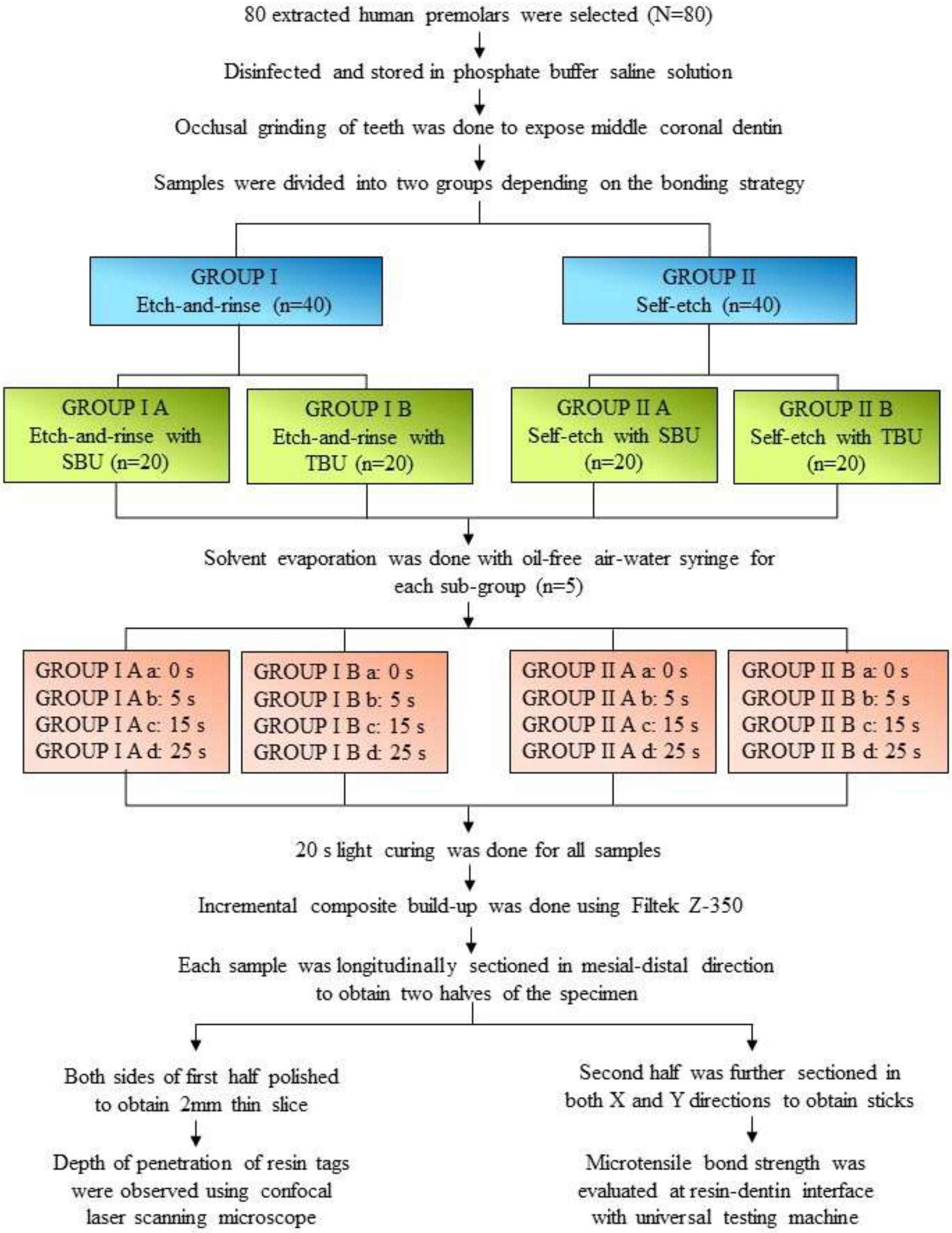
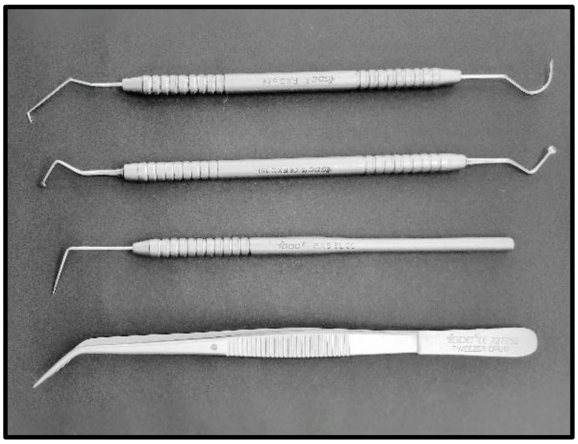
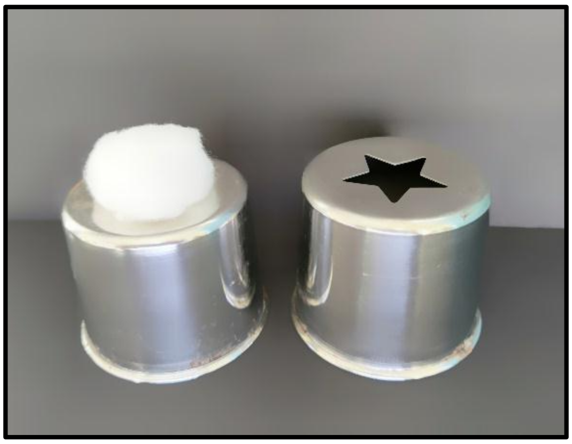


PLATE -I

ARMAMENTARIUM

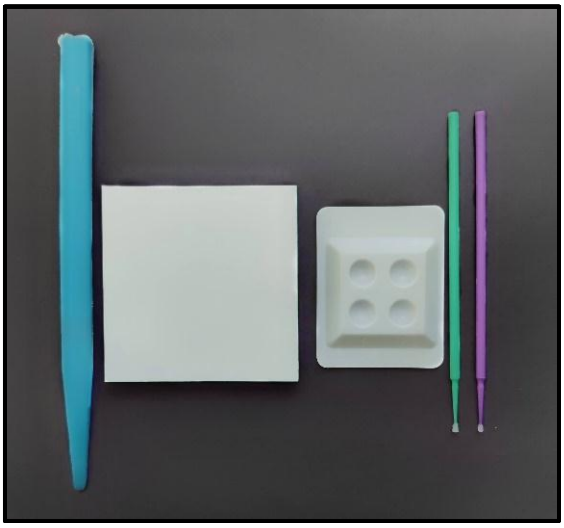


Hand Instruments
(GDC, India)



Cotton Holder & Waste Receiver
(GDC, India)

Digital Vernier Caliper
(Workzone tools, Germany)



Spatula, Mixing Pad, Dispenser, Microbrushes

PLATE -II

ARMAMENTARIUM

Micromotor with Straight Handpiece
(Saeshin, Korea)
Double sided Diamond Disc
(DSF, Germany)

Disposable Applicator Tips
(3M Espe St. Paul, USA)

Tofflemire Retainer (GDC, India)
Matrix Band, Mylar Strips

Composite Instruments
(GDC, India)

PLATE -III

ARMAMENTARIUM

LED Light Curing Gun
(Ivoclar Vivadent)

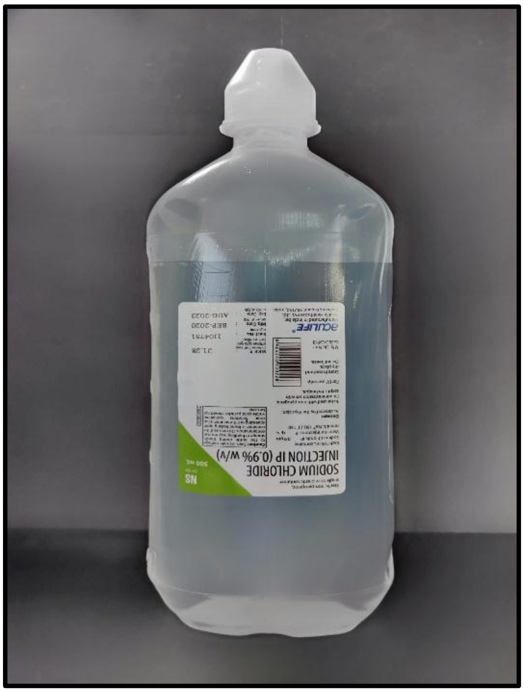
Air-Water Syringe
(Waldent, India)

Grinder and Polisher
(Buehler, Germany)

Isomet Sectioning Saw
(Buehler, Germany)

PLATE -IV

MATERIALS



Normal Saline
(Nirlife, India)

Rhodamine B Dye
(Loba Chemie, India)

Cyanoacrylate Adhesive
(Anabond, India)

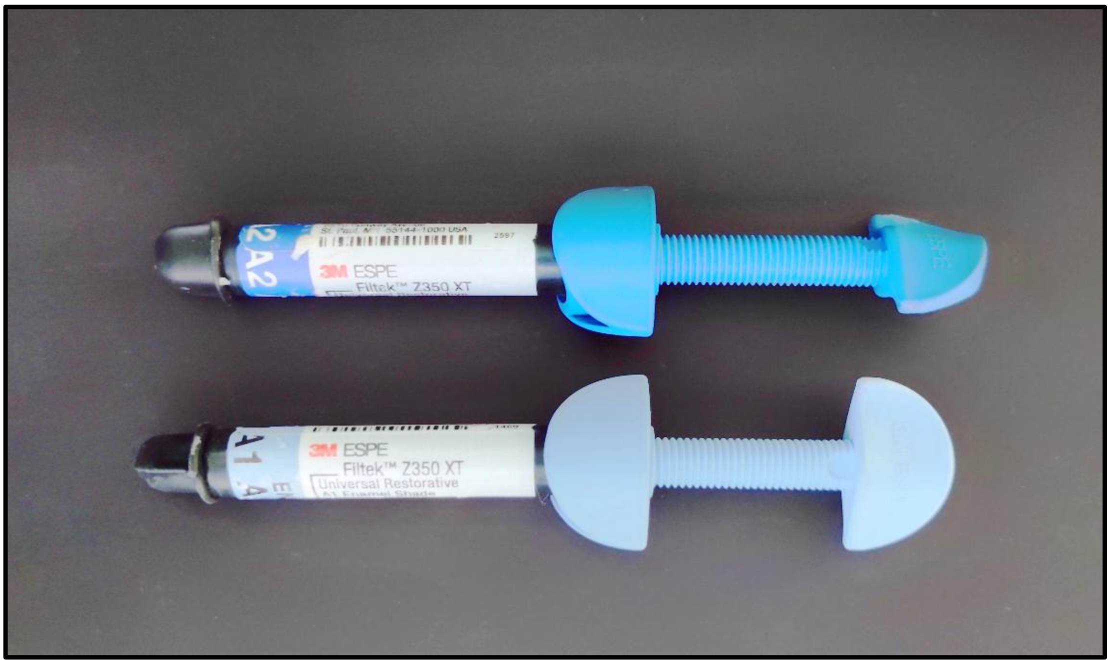
37% Phosphoric Acid Etchant
(Scotchbond, 3M Espe, USA)

PLATE -V

MATERIALS

Tetric N-Bond Universal
(Ivoclar Vivadent)

Single Bond Universal
(3M Espe, St. Paul, USA)

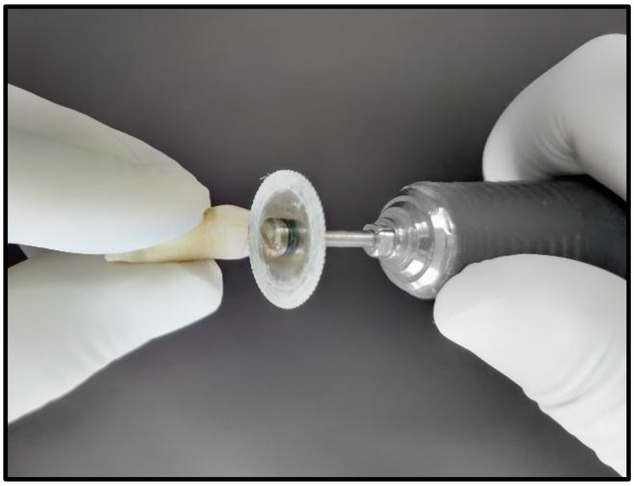


Filtek Z-350 XT Universal Restorative
(3M Espe, St. Paul, USA)

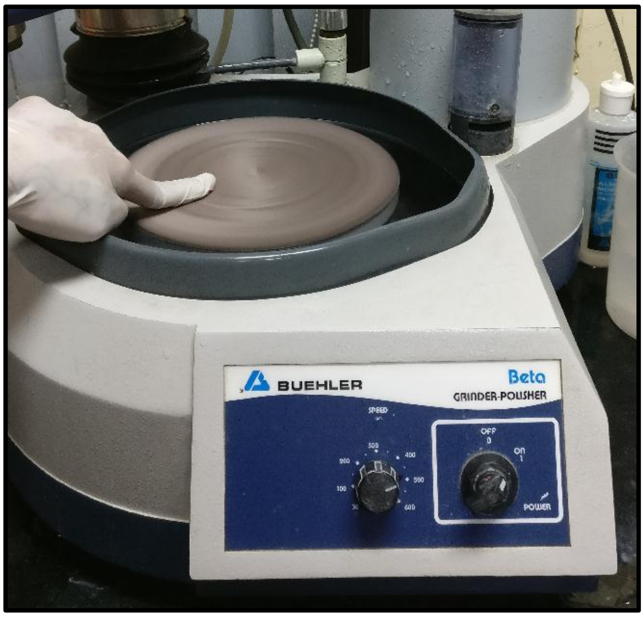
PLATE -VI

METHODOLOGY

Sample Size (N=80)



Occlusal grinding with diamond disc



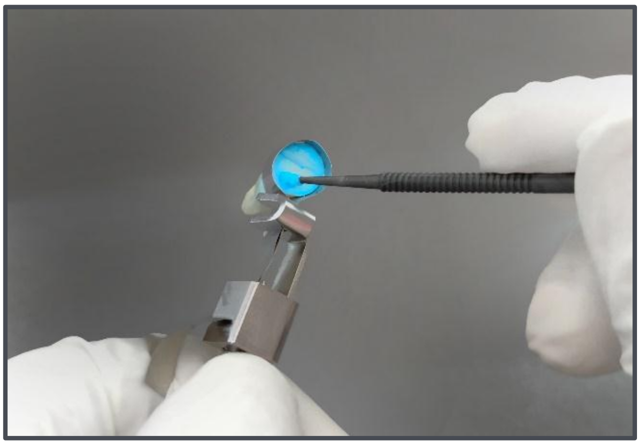
Wet polishing of coronal dentin with #600 SiC paper to standardize smear layer

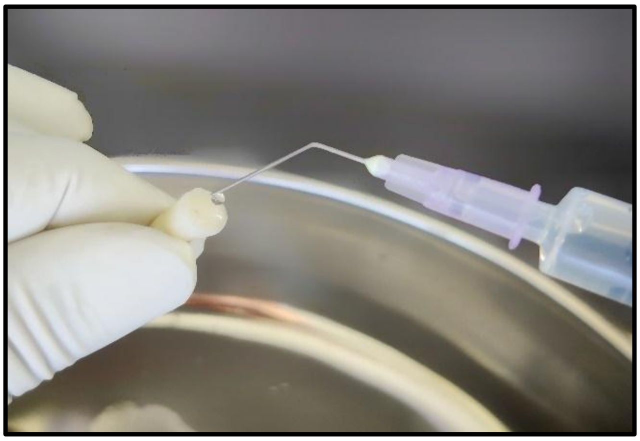
Occlusally ground and polished specimens (n=40)

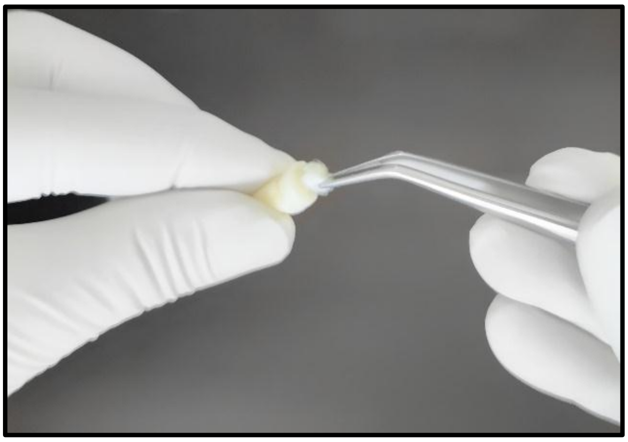
PLATE -VII

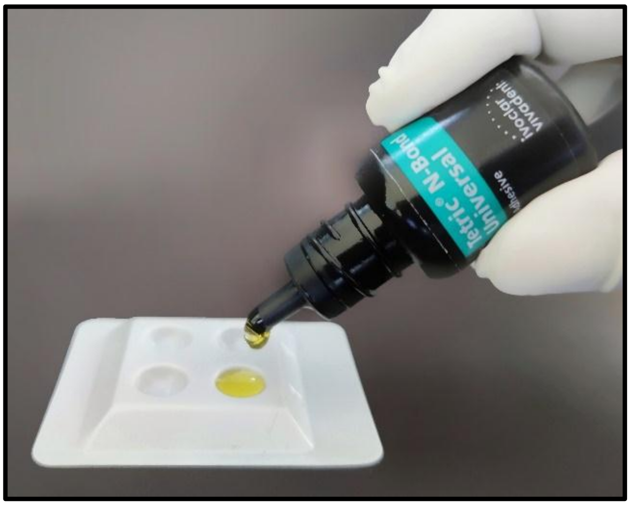
METHODOLOGY


Matrix band application

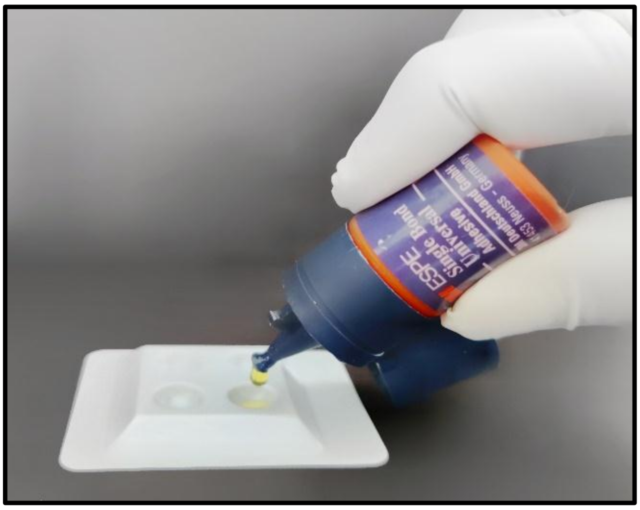

Application of Etchant for Etch-and-rinse group


Rinsing with water


Removal of excess water



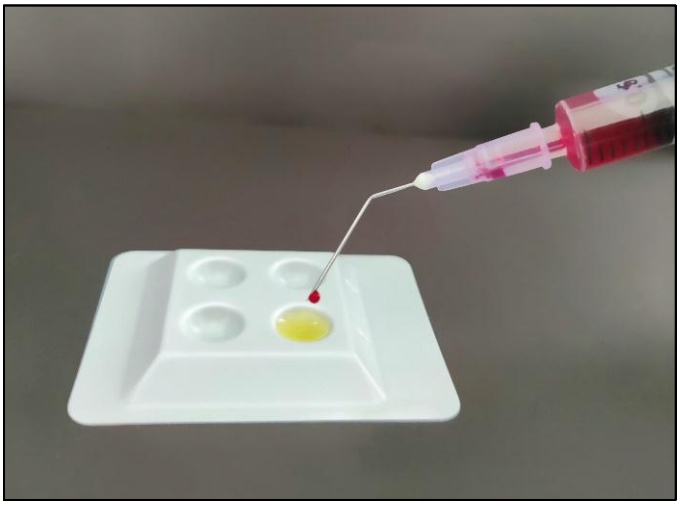
Dispensing of Tetric N-Bond Universal



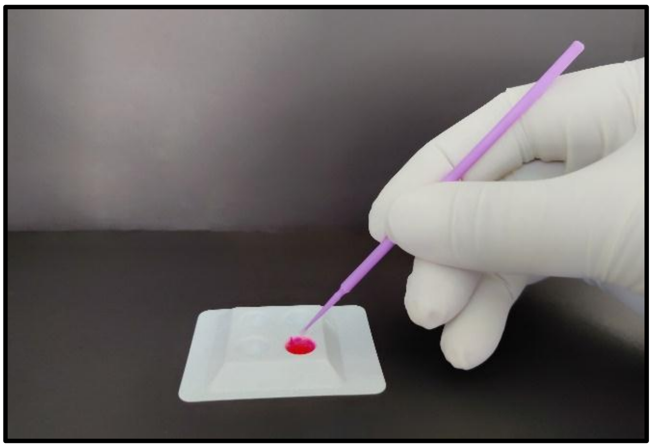
Dispensing of Single Bond Universal

PLATE -VIII

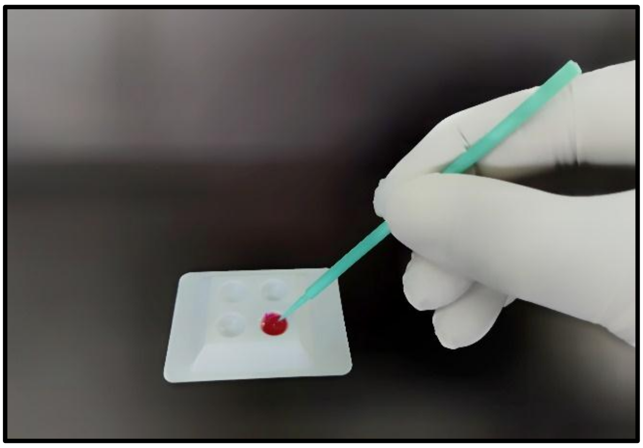
METHODOLOGY



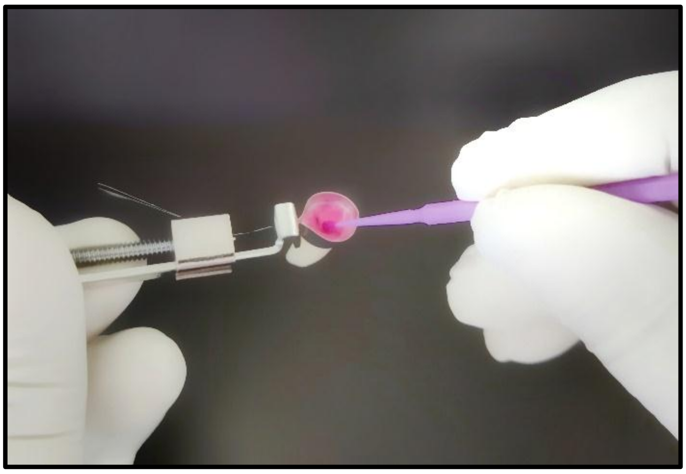
Dispensing of Rhodamine B Dye



Labelling of Tetric N-Bond Universal



Labelling of Single Bond Universal



Application of Bonding Agents

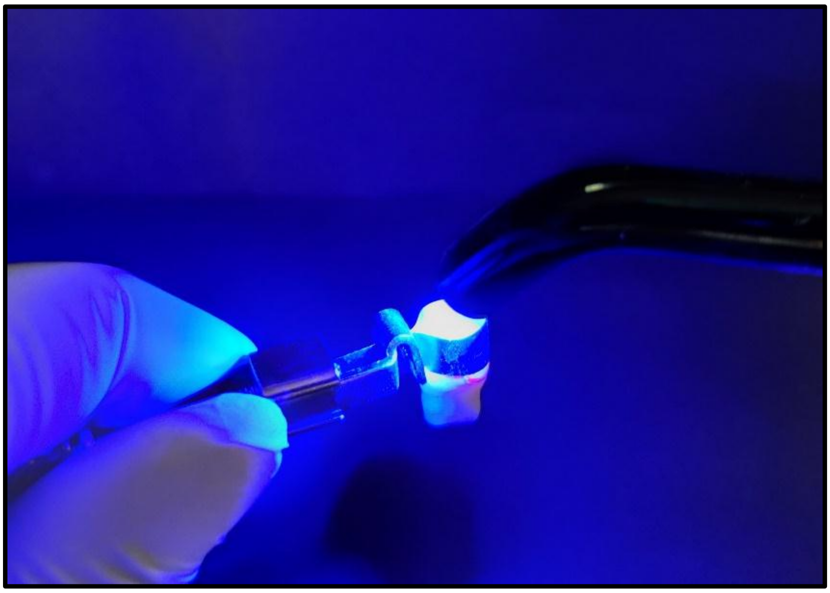
PLATE -IX

METHODOLOGY

Air-water syringe clamped at distance of 1.5 cm

Air Pressure adjusted to 1 Bar

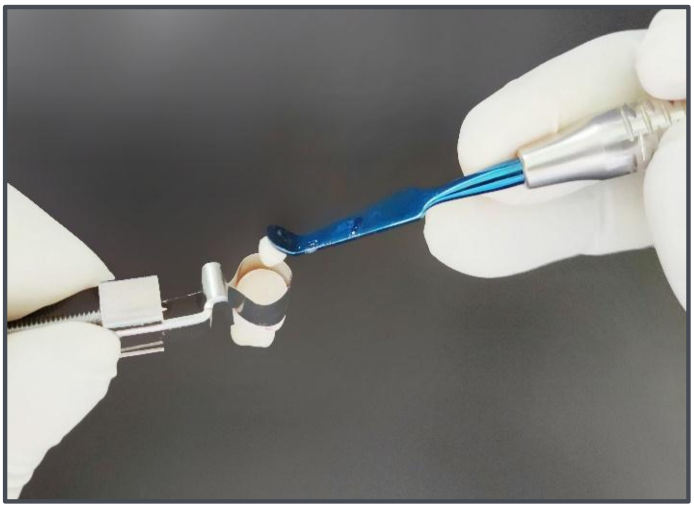
Solvent Evaporation



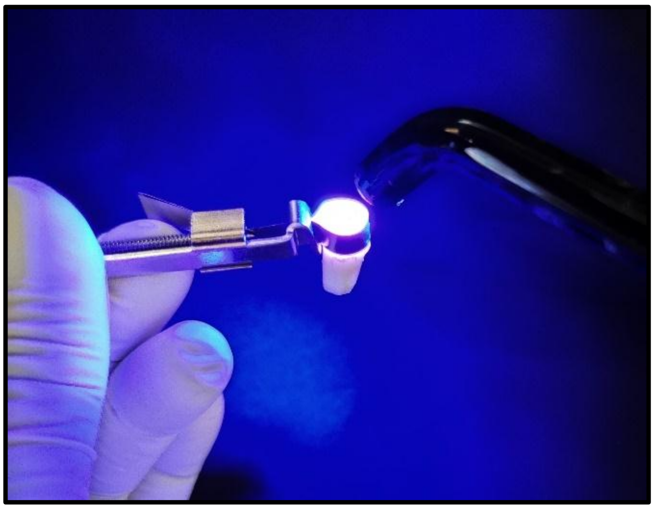
Curing of Bonding Agent

PLATE -X

METHODOLOGY



Incremental Composite restoration

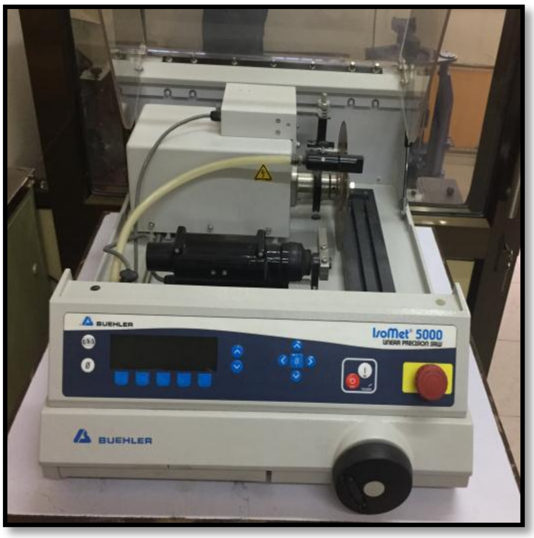


Curing of Composite

Final tooth specimen for each group

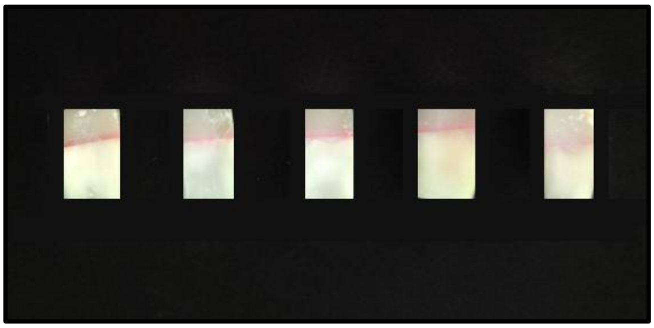
PLATE -XI

METHODOLOGY

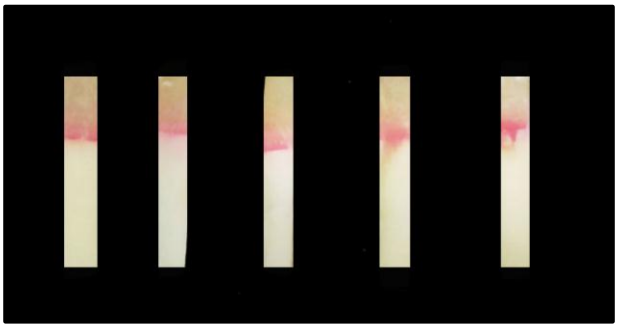


Sectioning of samples with Isomet Precision Saw
(Buhler, Germany)

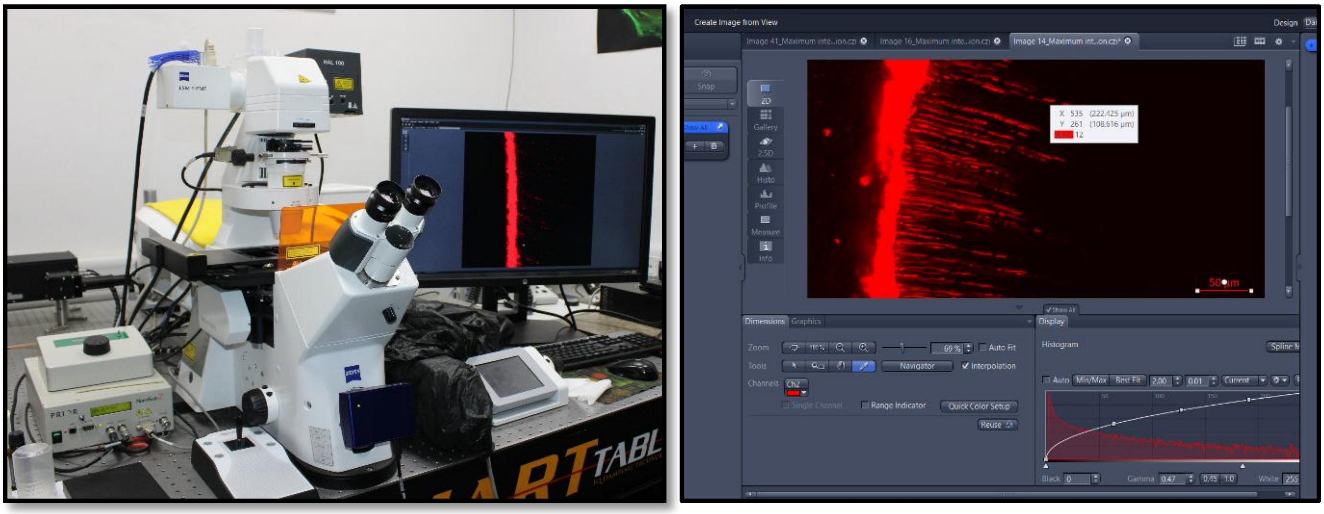
Polishing of samples
(Buhler, Germany)



Sectioned samples for CLSM testing



Sectioned samples for Microtensile Bond Strength testing



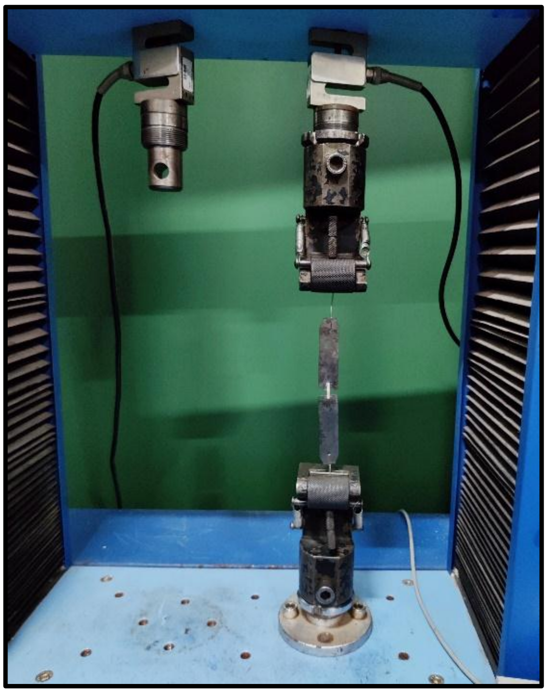
Confocal Laser Scanning Microscope
(LSM 510, ZIESS with LSM Software Zen 2007)

PLATE -XII

METHODOLOGY

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

Specimen bonded on jig

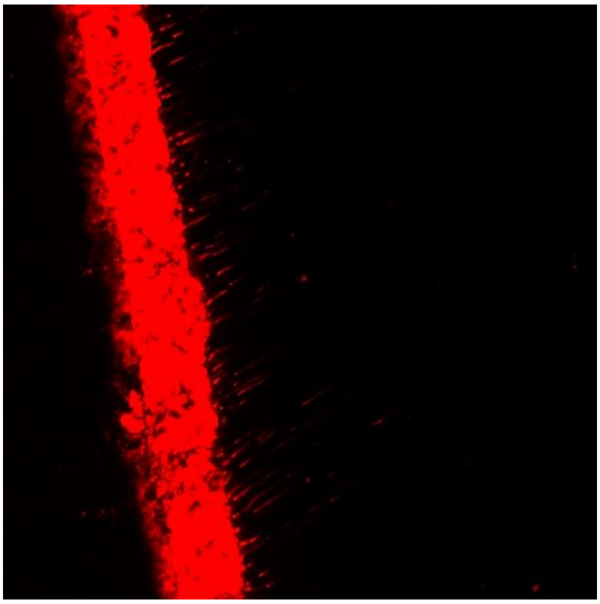


Microtensile Bond Strength testing on UTM
(ACME Engineers, Model no. UNITEST-10

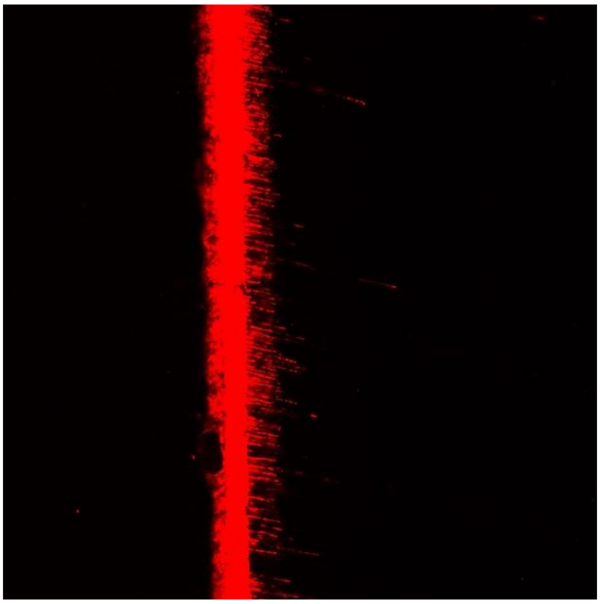
PLATE -XIII

CONFOCAL LASER MICROSCOPIC IMAGES

GROUP IA: ETCH AND RINSE STRATEGY WITH 3M ESPE SINGLE BOND UNIVERSAL



0 SECONDS
(CONTROL)



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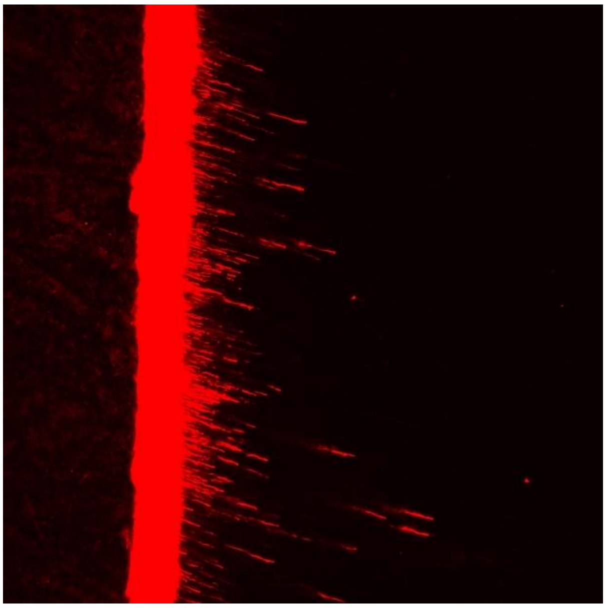
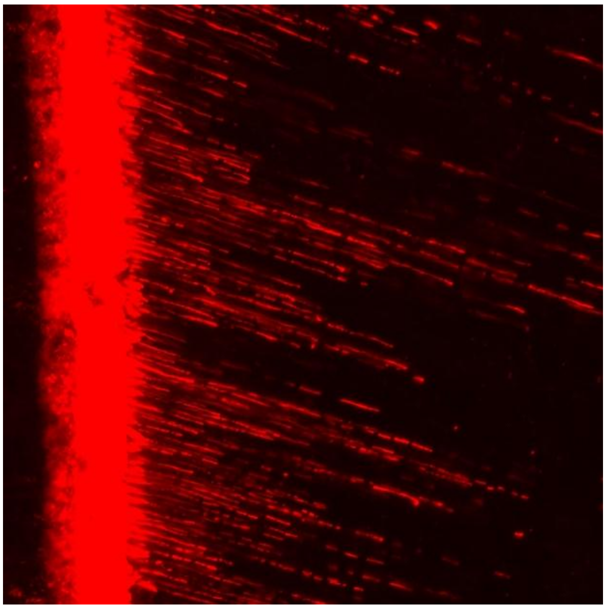


15 SECONDS

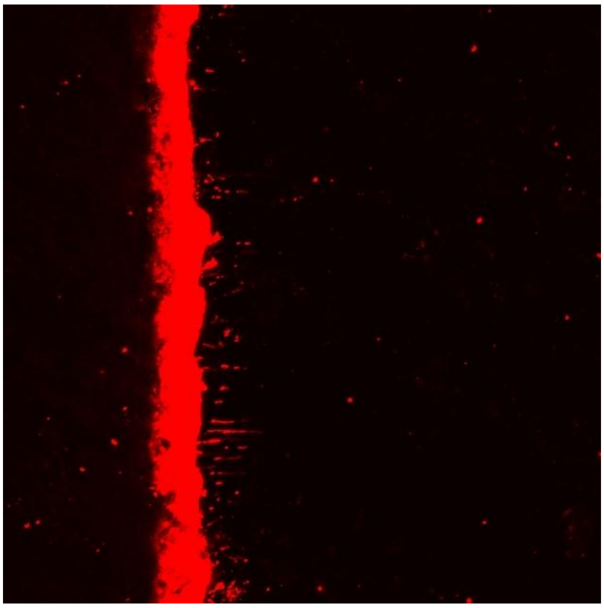


25 SECONDS

PLATE -XIV

CONFOCAL LASER MICROSCOPIC IMAGES

GROUP I B: ETCH AND RINSE STRATEGY WITH IVOCLAR TETRIC N-BOND UNIVERSAL



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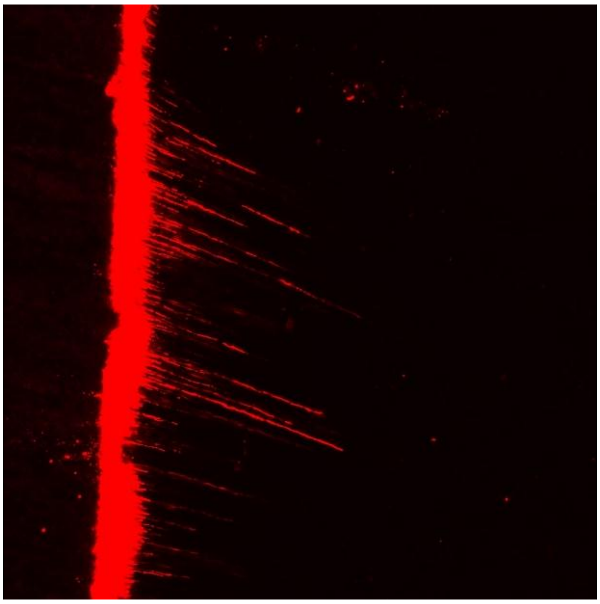
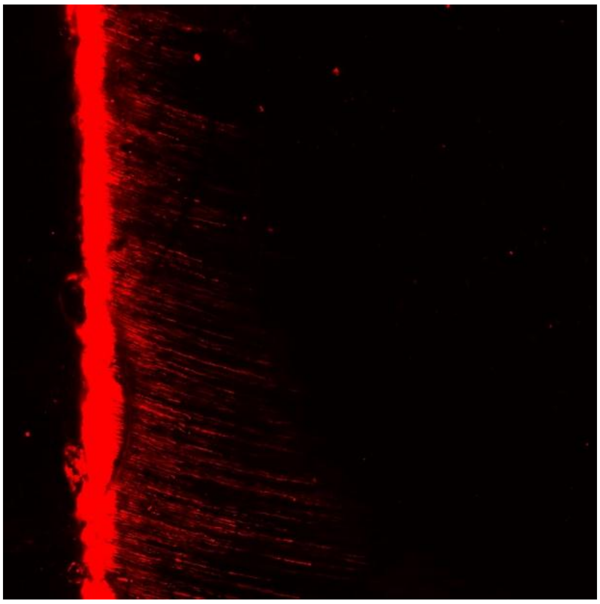
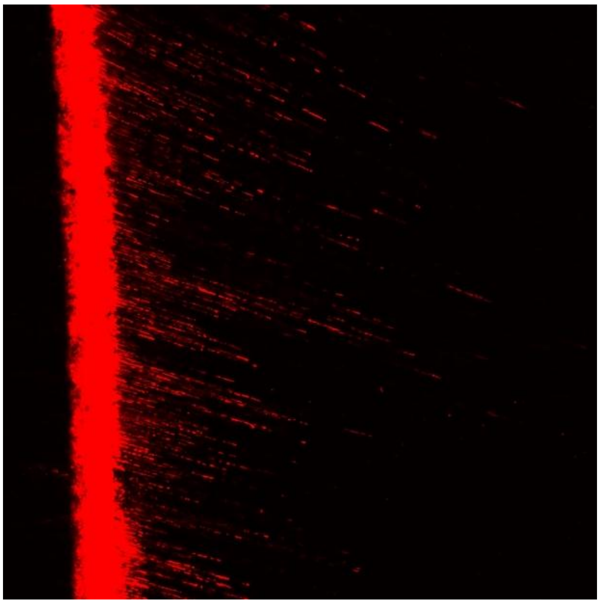
(CONTROL)



5 SECONDS



15 SECONDS

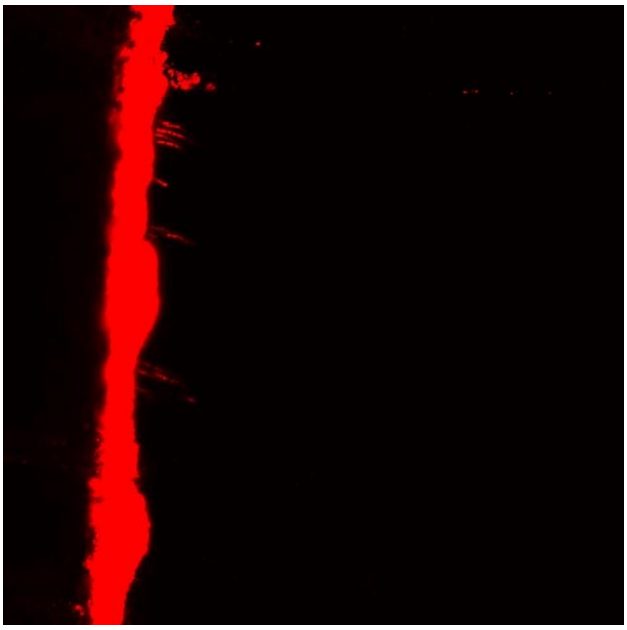


25 SECONDS

PLATE -XV

CONFOCAL LASER MICROSCOPIC IMAGES

GROUP IIA: SELF-ETCH STRATEGY WITH 3M ESPE SINGLE BOND UNIVERSAL



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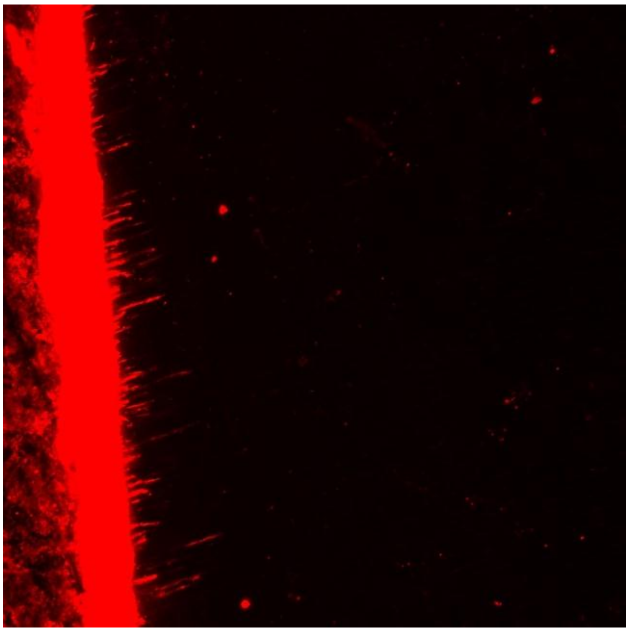
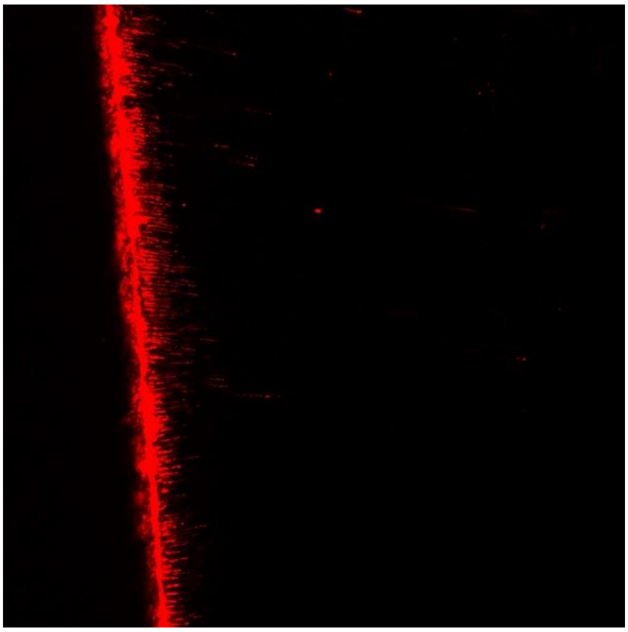
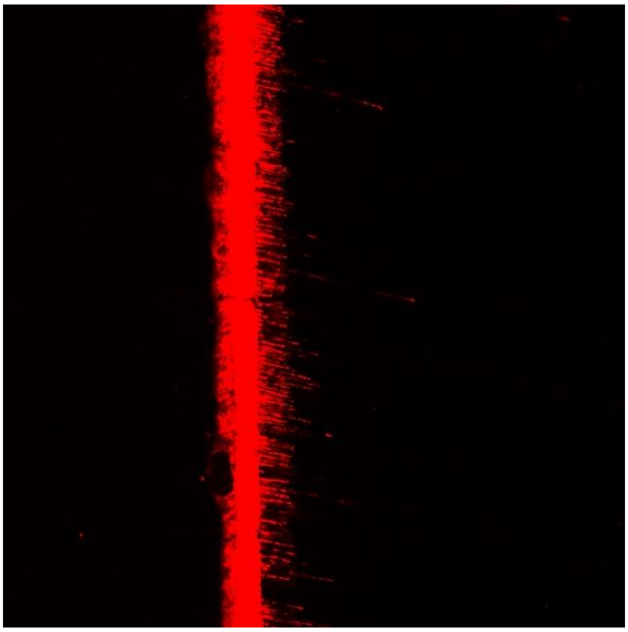
(CONTROL)



5 SECONDS



15 SECONDS

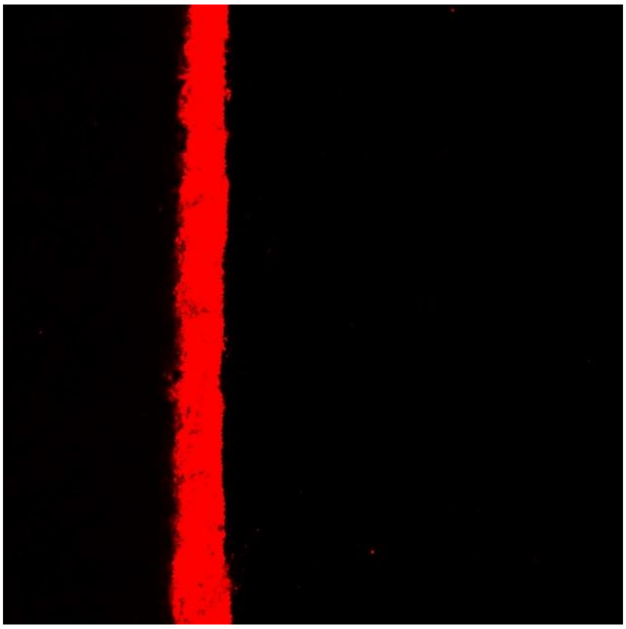


25 SECONDS

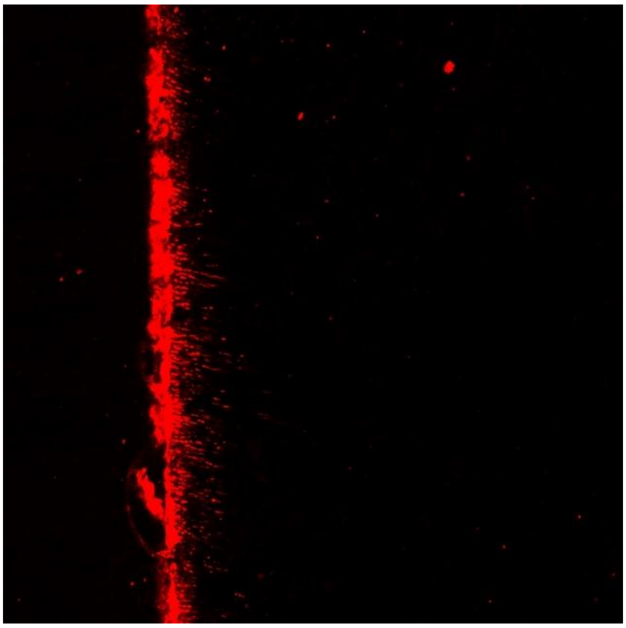
PLATE -XVI

CONFOCAL LASER MICROSCOPIC IMAGES

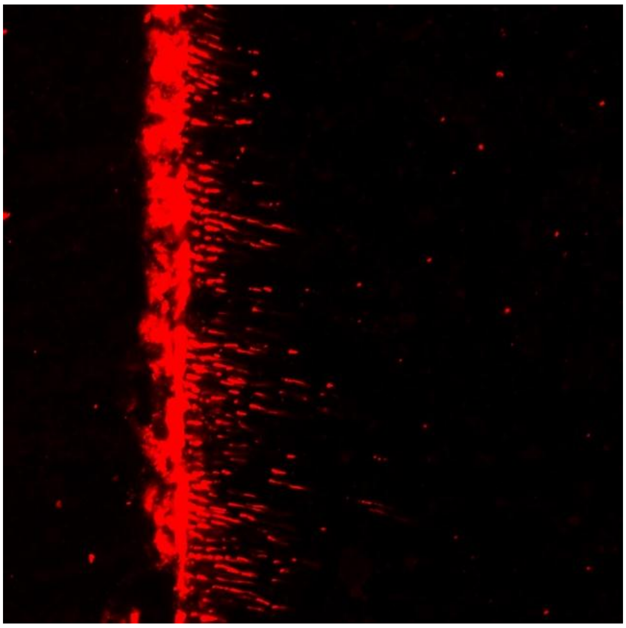
GROUP II B: SELF-ETCH STRATEGY WITH IVOCLAR TETRIC N-BOND UNIVERSAL



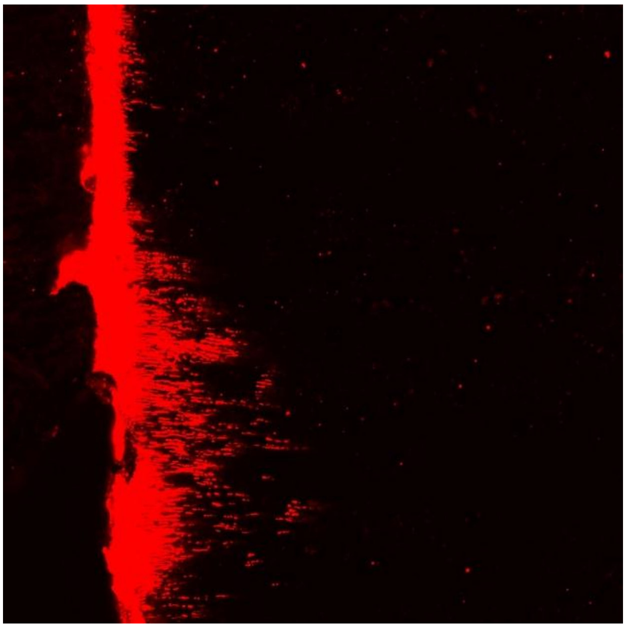
0 SECONDS
(CONTROL)



5 SECONDS



15 SECONDS



25 SECONDS

RESULTS

The present in vitro study was conducted to evaluate the depth of resin tags formed at the resin-dentin interface after use of two universal adhesives in self-etch and etch-and-rinse strategies in varying evaporation protocol by confocal laser scanning microscope and its relation to microtensile bond strength of adhesives to coronal dentin.

Depending upon the bonding strategies used for the universal adhesives, the samples were randomly divided into two broad groups:

Group I: Etch-and-rinse

Group II: Self-etch

Each group was further subdivided into two sub-groups according to the adhesive used:

1) **Sub-group I A :** Etch-and-rinse using 3M ESPE Single Bond Universal

2) **Sub-group I B :** Etch-and-rinse using Ivoclar Vivadent Tetric N-bond Universal

3) **Sub-group II A :** Self-etch using 3M ESPE Single Bond Universal

4) **Sub-group II B :** Self-etch using Ivoclar Vivadent Tetric N-bond Universal

Upon completion of bonding the adhesives in the aforementioned strategies with various solvent evaporation durations, the final groups were as follows:

Group	**Sub-group**	**Solvent Evaporation time**
GROUP I Etch-and-rinse	I A. Single Bond Universal	I Aa. 0 seconds (control)
		I Ab. 5 Seconds
		I Ac. 15 Seconds
		I Ad. 25 Seconds
	I B. Tetric N-bond Universal	I Ba. 0 seconds (control)
		I Bb. 5 Seconds
		I Bc. 15 Seconds
		I Bd. 25 Seconds
GROUP II Self-etch	II A. Single Bond Universal	II Aa. 0 seconds (control)
		II Ab. 5 Seconds
		II Ac. 15 Seconds
		II Ad. 25 Seconds
	II B. Tetric N-bond Universal	II Ba. 0 seconds (control)
		II Bb. 5 Seconds
		II Bc. 15 Seconds
		II Bd. 25 Seconds

Each tooth was sectioned longitudinally in a mesio-distal direction across the bonded interface with a microtome to attain two halves of each tooth specimen. One half was further sliced to obtain 2mm thick samples for evaluation of depth of resin tags under CLSM, while the other half was cut into samples of 1mm^2 sticks for evaluation of microtensile bond strength.

Statistical Analysis:

Licensed version of SPSS 20.0 (IBM Crop) was used for statistical analysis. The data on maximum penetration for of two adhesives viz., 3M ESPE Single Bond Universal and Ivoclar Vivadent Tetric N-bond Universal for each of the bonding strategies viz., Etch-and-rinse and Self-etch for three different evaporation times were obtained and summarized in terms of mean, standard deviation (SD) and median. Also, the micro-tensile bond strength of these samples with above exposures were obtained and summarized in terms of mean, SD and median.

The comparison of maximum penetration depth between two adhesives was carried out using t-test for independent samples for each exposure time. The analysis was performed for both the bonding strategies independently. On similar lines, the analysis was done for micro-tensile bond strength.

The analysis for the two parameters was also performed for each adhesive between two bonding strategies using t-test for independent samples. Further, the correlation between maximum penetration and bond strength for each bonding strategy and each adhesive was obtained using Pearson’s correlation coefficient. The analysis was performed for each exposure time independently.

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

The formulations used in the study are as below:

1. Measures of central tendency

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

Mean for a set of observations is given by

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

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

2. Measures of dispersion

- **Standard deviation** for a set of observations in given by

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

where x_i = observation on each object

n = number of objects

3. Statistical inference tests

- Student's t-test for independent samples

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

It is given by the formula:

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

where $\bar{x}_1$ and $\bar{x}_2$ are the means of sample observations of two different groups, μ_1 and μ_2 are the means of the respective populations from which the samples are derived, and *S* is the pooled sample standard deviation, which is given by:

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

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

Pearson's correlation

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

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

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

Overall Results:

The mean values and standard deviations of depth of penetration of adhesive and microtensile bond strength for the two adhesives in etch-and-rinse and self-etch strategies with different evaporation protocol have been described in Table.1 and Table.2 respectively.

The maximum depth of resin tag penetration (250.91 µm) was observed in Group I A at solvent evaporation duration of 25 s whereas the least depth of penetration of resin tag (45.97 µm) was observed in Group II A wherein no solvent evaporation was done (control group). (Table.3, Figure 1, and Figure 2)

The maximum microtensile bond strength (11.13 MPa) was observed in Group II A at solvent evaporation duration of 15 s whereas the minimum microtensile bond strength (2.14 MPa) was observed in Group II A wherein solvent evaporation was done for 0 s (control group). (Table.4, Figure 3, and Figure 4)

Higher values for depth of resin tags were obtained for Group I in which Single Bond Universal adhesive was used in etch-and-rinse strategy as compared to another group in which Tetric N-Bond Universal was used as bonding agent. The values for penetration depth of both Single Bond Universal and Tetric N-Bond Universal were higher in etch-and-rinse strategy as compared to self-etch strategy in all solvent evaporation protocols. (Table.1, and Table.3, Figure 1, and Figure 2)

Higher values for microtensile bond strength were obtained for Group II A in which Single Bond Universal adhesive was used in self-etch strategy at solvent evaporation duration of 15 s compared to Group I A in which Single Bond Universal was used in etch-and-rinse strategy at varying solvent evaporation protocols (0 s, 5 s, 15 s and 25 s). Statistically insignificant differences were observed in microtensile bond strength of Tetric N-Bond Universal in both the bonding strategies at all evaporation protocols. (Table.4, and Table.6, Figure 3, and Figure 4)

Analysis of depth of resin tag formation:

In order to determine the effect of solvent evaporation duration on depth of resin tag formation at resin-dentin interface, Student’s t-test was used for independent samples. Table 3 provides the comparison of maximum penetration depth between two adhesive i.e Single Bond Universal and Tetric N-Bond Universal in each group for samples exposed to varying evaporation times.

It is evident from the analysis that for the Etch-and-rinse strategy, the difference of mean penetration for control samples in Single Bond Universal was significantly greater than Tetric N-Bond Universal with a p-value < 0.0001. Also, for samples exposed to 5 sec evaporation time, the difference of mean penetration depth was statistically significantly higher in Single Bond Universal as compared to Tetric N-Bond Universal with a p-value of 0.024. For the other two exposure times, the difference of means was statistically insignificant ($p > 0.05$).

For the Self-etch category, for control samples, the difference of mean penetration depth of two adhesives was statistically significant with a p-value of 0.026 revealing greater depth of adhesive penetration in Tetric N-Bond Universal as compared to Single Bond Universal. Similarly, for evaporation time of 5 sec, the difference of means was significantly higher for Tetric N-Bond Universal with a p-value of 0.012. For samples exposed to evaporation of 15 sec, it was observed that the difference of means was statistically significantly higher for Tetric N-Bond Universal as compared to Single Bond Universal with a p-value of 0.006, while for those exposed to 25 sec evaporation, the difference was significant with a p-value < 0.0001.

In the present study, the maximum penetration depth for each adhesive type between two strategies at varying exposures was compared (Table.5). It was observed that for control group where solvent evaporation duration was 0 s, both Single Bond Universal and Tetric N-Bond Universal adhesives showed statistically significant difference of means between two strategies as indicated by p-values < 0.0001 and 0.031 respectively.

In samples exposed for 5 s, the difference of mean penetration depth was significant for Single Bond Universal with a p-value < 0.0001, while it was insignificant for Tetric N-Bond Universal adhesive ($p=0.075$). Similarly, for exposure of 15 s, the difference of mean penetration depth was significant for Single Bond Universal with a p-value of 0.001, while it was insignificant for Tetric N-Bond

Universal (p=0.121). For 25 s exposure category, both Single Bond Universal and Tetric N-Bond Universal showed statistically significant difference of means between two bonding strategies with p-values 0.008 and < 0.0001 respectively. (Table 5)

Analysis of depth of microtensile bond strength:

Student's t-test was used for independent samples in order to determine the effect of solvent evaporation duration on microtensile bond strength at resin-dentin interface. Table 4 provides the comparison of microtensile bond strength between two adhesives i.e Single Bond Universal and Tetric N-Bond Universal in each group for samples exposed to varying evaporation times.

It is evident from the findings of the present study that for Etch-and-rinse strategy, the difference of mean micro-tensile bond strength between two adhesives was statistically insignificant (p > 0.05) for every exposure. Further, for Self-etch type also, the difference of mean micro-tensile strength between two adhesives was statistically insignificant for every exposure.

Table 6 provides the comparison of mean micro-tensile bond strength for each adhesive type between two bonding strategies at varying exposure times. For control samples wherein no solvent evaporation was done, the mean micro-tensile strength for SBU showed statistically significant difference between two strategies with a p-value of 0.015; however, the means for Tetric N-Bond Universal differed insignificantly between two strategies (p=0.806). Further at 5 sec and 15 sec exposure times, the difference of mean strength of adhesive Single Bond Universal between two strategies was significant with p-values 0.023 and 0.022 respectively. (Table 6)

Thus, Single Bond Universal exhibited higher microtensile bond strength in etch-and-rinse strategy in both 0 s and 5 s solvent evaporation protocol. However, higher bond strength for SBU was observed in self-etch group at 15 s of solvent evaporation time as compared to etch-and-rinse group for the same evaporation protocol.

For Tetric N-Bond Universal, the difference of means was insignificant for both 5 sec and 15 sec exposures. For 25 sec exposure, the difference of mean strength for both the adhesive types was statistically insignificant (p > 0.05). (Table 6)

Analysis of relation between depth of resin tag penetration and microtensile bond strength:

Pearson's correlation coefficient was used to quantify the relationship between depth of adhesive penetration and microtensile bond strength.

Table 7 shows the correlation of maximum penetration depth of adhesive and corresponding micro-tensile bond strength for each bonding strategy and adhesive type for control samples. It is evident that for Etch-and-rinse bonding strategy and SBU, the correlation was weak positive (0.011) and statistically insignificant (p=0.986); while for Tetric N-Bond Universal, it was weak negative (-0.298) and statistically insignificant (p=0.627). It was observed that for the Self-etch bonding strategy, and for Single Bond Universal, the correlation between two parameters was weak negative (-0.184) and statistically insignificant (p=0.767); while for Tetric N-Bond Universal, it was weak positive (0.124) and statistically insignificant (p=0.842).

Table 8 shows the correlation of maximum penetration depth of adhesive and corresponding micro-tensile bond strength for each bonding strategy and adhesive type for samples exposed for 5 sec evaporation time. A low negative (-0.385) and statistically insignificant (p=0.522) correlation was observed for Etch-and-rinse bonding strategy and Single Bond Universal; while for Tetric N-Bond Universal, it was strong negative (-0.926) and statistically significant (p=0.024). For the Self-etch bonding strategy, and for Single Bond Universal, the correlation between two parameters was moderately positive (0.689), but statistically insignificant (p=0.199); while for adhesive Tetric N-Bond Universal, it was weak negative (-0.137) and statistically insignificant (p=0.826).

Table 9 shows the correlation of maximum penetration depth of adhesive and corresponding micro-tensile bond strength for each bonding strategy and adhesive type for samples exposed for 15 sec evaporation time. It was observed in Etch-and-rinse bonding strategy and Single Bond Universal, the correlation was moderately negative (-0.585) and statistically insignificant (p=0.301); while for Tetric N-Bond Universal, it was moderately negative (-0.541) and statistically insignificant (p=0.347). For the Self-etch bonding strategy and for Single Bond Universal, the correlation between two parameters was highly positive (0.871) but was statistically insignificant (p=0.054); while for Tetric N-Bond Universal, it was highly positive (0.764), but and statistically insignificant (p=0.132).

Table 10 shows the correlation of maximum penetration depth of adhesive and corresponding micro-tensile bond strength for each bonding strategy and adhesive type for samples exposed for 25 sec evaporation time. It was observed that for Etch-and-rinse bonding strategy and Single Bond Universal adhesive, the correlation was moderately positive (0.666) and statistically insignificant (p=0.221); while for Tetric N-Bond Universal, it was weakly positive (0.014) and statistically insignificant (p=0.982). For the Self-etch bonding strategy, and for SBU adhesive type, the correlation between two parameters was weakly positive (0.026), and statistically insignificant (p=0.976); while for Tetric N-Bond Universal, it was weakly negative (-0.047), and statistically insignificant (p=0.941).

DISCUSSION

The advent of adhesives and the understanding of their interaction with dental hard tissues have recognizably become landmarks for the practice of modern restorative dentistry. Adhesive systems have progressed from the largely ineffective generations of the 1950s and early 1970s[10-11] to the relatively successful total- and self-etching systems available contemporarily. Universal adhesives are the latest simplification added to the generations of bonding agents categorized as single-bottle, no-mix, adhesive systems that can be used in total-etch, self-etch, or selective-etch mode depending on specific clinical situations and preferences of the clinician **(Alex et al. 2015[49]).**

Although adhesion to enamel has remained consistently simple and predictable since the introduction of the acid-etch technique[4], the pursuit for establishing reliable adhesion to dentin prevails to be a challenging procedure due to its dynamic compositional differences and complex histology.[1-3]

Since the intrinsic wetness of dentin substrate is a reality **(Van Meerbeek et al. 2003[12])**, incorporation of hydrophilic monomers into the composition of dentin bonding system to reduce associated technique-sensitivity has been practiced over decades.

Dissolution of monomers in a solvent enhances extent of diffusion in the porous conditioned substrate, especially in dentin due to its hydrophilic nature. Water, ethanol, and acetone are presently the most common solvents added into adhesive systems **(Reis et al. 2003[12]).**

Solvents critically ensure diffusion of monomers into the humid demineralized dentin substrate and are pivotal in removal of moisture from the substrate during evaporation. However, the excess solvent must be eliminated from adhesive after diffusion as remaining solvent may jeopardize polymerization due to the dilution of monomers resulting in voids and increased permeability of the adhesive layer **(Ikeda et al 2005[13]).** Therefore, after bonding resin applications, air stream is used to evaporate the remaining solvent to facilitate formation of a uniform layer of adhesive on the substrate.

Previous studies have demonstrated that increasing the solvent evaporation time results in higher bond strengths values **(Jacobsen et al., 2006[14]; Sadr et al., 2007[15]; Furuse et al., 2008[22])** and has a significant effect on the mechanical properties of the adhesive upon setting **(Ikeda et al., 2008[16]).** On the other hand, studies have also reported that prolonged drying is very detrimental to bond strength and causes catastrophic failure of adhesion **(Chiba et al. 2006)[23].**

Thus, contemporarily there is no consensus regarding the appropriate solvent evaporation duration for universal adhesives in both etch-and-rinse and self-etch strategies to achieve predictable bonding. Also, there is scarcity of research on effects of extended solvent evaporation of these adhesive systems upon quality of hybrid layer formation and its inter-relation to microtensile bond strength in the two aforementioned bonding strategies.

Hence, the following study was conducted to evaluate the quality of hybrid layer of two different universal adhesives in self-etch and etch-and-rinse strategies after varying evaporation protocols using Confocal Laser Scanning Microscopy (CLSM) and its relation to microtensile bond strength.

Eighty extracted human maxillary and mandibular premolars were selected for the study. The teeth were cleaned, disinfected and stored as per the recommendations and guidelines laid down by OSHA and CDC[50]. The selected teeth were stored in phosphate buffer saline solution for not more than 12 weeks as suggested by **Jameson MW et al (1994).** They observed that storage media and time of the specimen storage affects 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.[51]

The data published on micro-tensile bond strength by **Luque-Martinez IV et al. (2014)[58]** was referred to obtain the sample size for the proposed study. One of the aims of the study was to evaluate the difference of mean bond strength between two bonding strategies viz., Etch & Rinse and Self-Etch for each adhesive agent and for different solvent evaporation durations. The data from published study resulted into effect sizes ranging between 1.82 – 5.57. In order to achieve the minimum effect size of 1.82, which is the standardized mean difference between the mean bond strength of two strategies, for any air-dry exposures, a sample of 5 teeth per group are required that can provide the desired effect with 80% power and 95% confidence. Accordingly, considering the study design, the total number of samples required were **80**.

The formula used for estimating the sample size was:

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

where, $Z_{1-\alpha/2}$ and $Z_{1-\beta}$ are the standardized value for α=5% error (1.96) and β=20% (0.842) and ES is the effect size, which is given by

$$ES = \frac{\overline{x}_1 - \overline{x}_2}{s}$$

where *s* is the pooled standard deviation for two groups.

Premolars present a unique combination of occlusal dynamics, structural loading and anatomical design.[71] Also, they are most frequently extracted for orthodontic purposes, which enables easier availability of these teeth for in-vitro research. Therefore, premolars were selected for this study.

The majority of ultra-morphological anatomical studies analyze the coronal dentinal substratum. **Cagidiaco and Ferrari[70]** demonstrated how the anatomy of the coronal dentin is characterized by different density, diameter, and orientation of dentinal tubules in different cavity preparation cutting planes. Therefore, in the present study, the occlusal enamel was removed to expose the middle coronal dentin and further wet polishing was done with 600 grit SiC paper (Struers LaboPol-4. Struers, Copenhagen, Denmark) for 60 seconds to standardize the smear layer. This led to standardization and uniformity in samples.[40] (Plate-VI)

Van Meerbeek, et al. classified bonding agents based on the interaction of adhesives with dental substrates as etch-and-rinse and self-etch adhesives. While Etch & rinse technique involves the use of 30-40% phosphoric acid etchant to remove the smear layer, the self-etch approach is based on the use of non-rinse acidic monomers that simultaneously condition and prime dentin and enamel.[8]

In the present study, the samples were randomly divided into two broad categories according to the bonding strategy used:

1) Group I: etch-and-rinse

2) Group II: self-etch

More recently, a new generation of bonding agents has been introduced as the universal or multi-mode adhesives[9], which may be used either as etch-and-rinse or as self-etch adhesives.

Previous research has shown that application of an etching step prior to universal adhesives improves their dentine penetration but does not affect their bond strength to dentine. **Wagner et al.** in **2014** reported that similar bond strength values were observed for the universal adhesives regardless of the two application modes, which makes them reliable for working under different clinical conditions.[41]

Although several commercial brands are available in the market, most of the evidence in literature reports findings from Single Bond Universal (3M ESPE, St. Paul, MN, USA), which was one of the first to mention its “universal” character.[53] Currently, abundant scope exists for evaluating other multi-mode adhesives, especially applied to the dentin using different bonding strategies. Tetric N-Bond Universal is a recently introduced single component, light-cured adhesive indicated for direct and indirect bonding procedures.

Tetric N-Bond Universal is similar in its solvent composition with Single Bond Universal as both the bonding systems are categorized as ethanol-water based adhesives. However, the two adhesives differ in their monomeric composition as Single Bond Universal contains HEMA, MDP and Vitrebond Copolymer whereas Tetric N-Bond Universal matrix is based on mixtures of hydrophilic (HEMA), hydrophobic and intermediate monomers (Bis-GMA).[68] Thus, these two universal adhesive systems (Single Bond Universal and Tetric N-bond Universal) were used in the present study to evaluate their efficiency in both the bonding strategies.

Thus, the two broad categories of the aforementioned groups were further subdivided based on the adhesive used as follows:

1. Group I: etch-and-rinse

Sub-group I A: etch-and-rinse with Single Bond Universal

Sub-group I B: etch-and-rinse with Tetric N-Bond Universal

2. Group II: self-etch

Sub-group II A: Self-etch with Single Bond Universal

Sub-group II B: Self-etch with Tetric N-Bond Universal

When using the etch-and-rinse strategy, the first step primarily involves the application of a demineralizing acid gel to the dental substrate which allows removal of the smear layer and exposure of the collagen fibrils in dentine.[54] Acid etching results in washout of inorganic minerals from the tooth surface, hence the duration of its exposure to dental hard tissues can affect the underlying physical and mechanical surface properties as well. Previous studies found a direct correlation between extended acid etching time and demineralization depth, resulting in a poorly infiltrated hybrid layer, increased surface roughness and reduced bond strength.[73] Therefore, in the current research, in **etch-and-rinse** group, the exposed dentin surfaces were etched with 37% phosphoric acid for 15 s and rinsed with water for 30 s as per the manufacturer's recommendations.

Jayaprakash et al. in **2010** evaluated the effect of surface moisture on dentinal tensile bond strength and reported that blot drying with a tissue paper provided consistently better bond strength. Thus, the excess water was removed from the dentin surface with an absorbent paper in this study.

On the contrary, no phosphoric acid etching was done in the **self-etch** group in the present study. Universal adhesives were directly applied on the exposed dentin surfaces with gentle rubbing motion for 20 s as per the manufacturer's instructions.

Previous studies[75] have reported the benefits of active application of adhesive by rubbing it on tooth substrate for optimal dentin bond performance and durability with self-etch adhesives. Increased dentin bond strength with active application has been suggested to be due to the stirring of adhesive which induces solvent evaporation, resulting in a higher rate of resin monomer incorporation inside the smear layer. Furthermore, the nano layering of calcium-salt formed from hydroxyapatite and the functional monomer is significantly greater with active application than with inactive application.

Burrer et al. reported that an adhesive application time of at least 20 s is mandatory to achieve sufficient dentin bond strength for universal adhesives as it ensures adequate resin infiltration, thus emphasizing the importance of strict adherence to the minimum recommended application time.[74]

Peutzfeldt et al. emphasized that it is imperative to follow the manufacturer's recommendation to achieve an optimum bonding to dentin with dentin adhesives. For the most reliable result, the clinical procedure recommended by the manufacturer should be followed meticulously as deviations from the manufacturer's protocols significantly reduced bond strength to dentin.[56]

Solvents play a crucial role in adhesive infiltration into the wet dentin substrate. The monomer interdiffusion has been demonstrated as the fundamental mechanism in achieving effective dentine bonding. Therefore, the solvents should be completely removed during clinical application of the adhesive with air-drying to prevent jeopardization of polymerization reaction.[36]

Previous research demonstrates that increasing the solvent evaporation time results in higher bond strengths values. [21,15,22,16]

Spreafico et al. (2006) observed that the use of air-jet after the application of adhesive improves the evaporation of solvent and water, eventually reducing the thickness of adhesive layer and formation of a uniform layer of adhesive on the substrate[20]. In the present study, each adhesive (Single Bond Universal and Tetric N-Bond Universal) was applied as etch-and-rinse adhesive or as self-etch adhesive; and with four adhesive solvent evaporation times (0 s, 5 s, 15 s, and 25 s).

Thus, the final categorization of samples was done accordingly into the following groups:

1. Group I: etch-and-rinse

A. Sub-group I A: etch-and-rinse with Single Bond Universal

- Sub-group I Aa: etch-and-rinse with Single Bond Universal with no solvent evaporation (control group)
- Sub-group I Ab: etch-and-rinse with Single Bond Universal with solvent evaporation duration of 5 s
- Sub-group I Ac: etch-and-rinse with Single Bond Universal with solvent evaporation duration of 15 s
- Sub-group I Ad: etch-and-rinse with Single Bond Universal with solvent evaporation duration of 25 s

B. Sub-group I B: etch-and-rinse with Tetric N-Bond Universal

- Sub-group I Ba: etch-and-rinse with Tetric N-Bond Universal with no solvent evaporation (control group)
- Sub-group I Bb: etch-and-rinse with Tetric N-Bond Universal with solvent evaporation duration of 5 s

- Sub-group I Bc: etch-and-rinse with Tetric N-Bond Universal with solvent evaporation duration of 15 s
- Sub-group I Bd: etch-and-rinse with Tetric N-Bond Universal with solvent evaporation duration of 25 s

3. Group II: self-etch

A. Sub-group II A: Self-etch with Single Bond Universal

- Sub-group II Aa: Self-etch with Single Bond Universal with no solvent evaporation (control group)
- Sub-group II Ab: Self-etch with Single Bond Universal with solvent evaporation duration of 5 s
- Sub-group II Ac: Self-etch with Single Bond Universal with solvent evaporation duration of 15 s
- Sub-group II Ab: Self-etch with Single Bond Universal with solvent evaporation duration of 25 s

B. Sub-group II B: Self-etch with Tetric N-Bond Universal

- Sub-group II Ba: Self-etch with Tetric N-Bond Universal with no solvent evaporation (control group)
- Sub-group II Bb: Self-etch with Tetric N-Bond Universal with solvent evaporation duration of 5 s
- Sub-group II Bc: Self-etch with Tetric N-Bond Universal with solvent evaporation duration of 15 s

- Sub-group II Bd: Self-etch with Tetric N-Bond Universal with solvent evaporation duration of 25 s

Kumar et al. (2016) suggested that oil contamination of any bonding agent while achieving evaporation of solvents provides an unpredictable clinical result due to potential bond failure.[57] Thus, after application of universal adhesives, evaporation of solvent was done with oil-free air-water syringe in the current research.

El-Askary et al. (2011)[59] investigated the effect of air-drying pressure and distance on bond strength of adhesives and suggested that when the drying distance is increased, the resultant adhesive layer is porous with no evidence of hybrid layer formation or resin tag infiltration. They also reported that increasing the solvent evaporation pressure at 2.6 and 4 bar at 2 cm distance produces a thin or virtually non-existent adhesive layer.

Therefore, in the present study, the air-drying pressure was adjusted to 1 Bar using a pressure regulator[58] and the distance was fixed by measuring the distance from the tip of the air-way syringe to the flat dentin surface, and the distance was maintained by clamping the syringe at a distance of 1.5cm from dentin surface.[59] (Plate-IX)

Following curing of the adhesive, each tooth was restored with Filtek Z350 XT Universal Restorative (3M ESPE, St. Paul, USA), a light cured packable composite resin. The composite was incrementally placed on dentin surfaces of all the samples, with each increment being approximately 1 mm in thickness and light cured for 20 s.

All samples were stored in distilled water for 24 hours after completion of the restorative procedure. Each tooth was sectioned longitudinally in a mesio-distal direction across the bonded interface with a microtome precision (Isomet, Beuhler, Germany) to attain two halves of each tooth specimen. (Plate-XI)

The ability of the adhesive systems to infiltrate into dental substrates and form high-quality hybrid layer is one of the primary requirements for adequate bonding.[29] Although interpretation of resin tag formation is speculative, several researchers have utilized the morphology, length, and density of resin tags for evaluation of the efficiency of adhesive systems.[70] Thus, the present study aimed at evaluating the depth of resin tag penetration into dentin to determine the quality of hybrid layer formed by the two adhesives used in this research.

Evaluation of the resin dentin interface (RDI) is currently done by several microscopic techniques 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 at relative low magnification as 100× using fluorescent Rhodamine–marked primers or bonding agents.[26]

Watson and Boyde[25] first described the use of fluorescent confocal microscopy for analysis of the interface of adhesive materials and tooth structure. They advocated the use of fluorescent dyes, mixed into components of an adhesive system, to highlight the bonded interface. In the present study, 0.1% Rhodamine B dye was mixed with equal drops of the two universal adhesives (Single Bond Universal and Tetric N-Bond Universal) used.

CLSM 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.[17]

Thus, in the current research CLSM was preferred over other microscopic techniques 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.

The microtensile bond strength test is currently considered as a versatile and standard bond strength testing method of adhesive interfaces. The test itself together with the morphological and spectroscopic investigations has been constantly contributing to improve resin/dentin adhesion and has proven to have a greater discriminative power than the traditional macro-shear test.[60]

Since its introduction in 1994, the original method for specimen preparation for microtensile bond strength test involved trimming of tooth at resin/dentin interface to obtain a rectangular cross-section of tooth resembling hourglass shape.[18] However, the trimming method has certain demerits due to its technique sensitivity, as it increases the chances of specimen damage by creating defects, flaws and additional stress which may facilitate premature failures.[76]

Thus, to optimize stress distribution and prevent crack propagation at the critical adhesive interface, stick shaped cylindrical cross-sectional specimens were produced to modify the traditional hourglass design. Basically, the modified testing method, also known as non-trimming technique, employs stick shaped specimens with 1 mm × 1 mm cross-sectional area.[18]

The main reasons for using the modified technique for specimen preparation in the present study included the ease of sample preparation with non-trimming method as it is less technique sensitive and allows uniform stress distribution at the adhesive interface.

In the present study, depth of penetration of resin tags was compared after the use of different solvent evaporation protocols in self-etch and etch-and-rinse strategies, at different regions of tooth under CLSM. Also, its relation to microtensile bond strength was evaluated.

1. Effect of different solvent evaporation protocols on depth of resin tag penetration in etch-and-rinse strategy:

The formation of intimate mechanical interlocking between partially demineralized dentin and dental adhesives, also called as resin-dentin interdiffusion zone as stated by **Nakabayashi et al.** is the benchmark for ultimate bonding.[5]

The infiltration of resin into the interfibrillar spaces of acid pretreated collagen meshwork results in formation of resin tags into the dentinal tubules creating the hybrid layer at resin-dentin interface. Resin tag development into the opened dentin tubules additionally contributes to the eventual bond.[43]

In the present study, maximum depth of resin tag penetration was observed with the etch-and-rinse approach as compared to self-etch approach irrespective of the universal adhesive used. The mean depth of penetration of adhesive in etch-and-rinse strategy was found to be highest **(202.57 µm)** in Single Bond Universal Group with solvent evaporation time of 25 s as compared to other groups and the least depth of penetration **(60.64 µm)** was observed in the control group of Tetric N-Bond Universal wherein no solvent evaporation was performed.

These results are in accordance with findings of **Baweja et al. (2007)**[61] who evaluated the resin tag formation and hybrid layer of total etch technique in comparison with self-etching primers with three different pH and observed statistically greater penetration depth in total etch group when compared to self-etching primers.

The smear layer appears to be a major challenge for the hybrid layer and resin tag formation in etch-and-rinse approach. Unless the smear layer is removed, neither resin tag formation nor hybridization can occur.[62]

In the present study, achievement of greater depth of resin tag penetration in etch-and-rinse strategy can be elucidated based on removal of smear layer by etching with phosphoric acid and rinsing-off the reaction products with water. This results in complete removal of smear layer and smear plugs from the dentin, leading to increased dentin permeability and better infiltration of resin, eventually aiding in the greater penetration of the resin tags and thick hybrid layer.

For the Etch-and-rinse strategy in the present study, the difference of mean penetration for control samples of both the universal adhesives was significantly different with a **p-value < 0.0001**. Also, for samples exposed to 5 sec evaporation time, the mean penetration depth of Single Bond Universal was found to be significantly greater than Tetric N-Bond Universal. (**p-value of 0.024**) For the other two exposure times, the difference of means was statistically insignificant ($p > 0.05$).

This difference can be explained due to compositional variation of monomers present in the two universal adhesives used. Vitrebond copolymer is a methacrylate-modified polyalkenoic acid copolymer present in Single Bond Universal (3M ESPE) which is resistant to changes in the humidity of dentin surface. It is responsible for rehydrating the collagen fibers that remain stable in aqueous medium, infiltrating the adhesive system throughout the entire demineralized depth in prior acid etching.[63] Therefore, it may improve the penetration of Single Bond Universal adhesive into the dentinal tubules in the etch-and-rinse application mode.

The penetration capability of adhesives may be closely dependent on their constituents, such as the amount of water, type of solvent, presence or absence of inorganic fillers, and hydrophilic or hydrophobic resin monomers.

Van Landuyt et al in **2007** suggested that water is essential to ionize the acidic monomers and trigger the demineralization process. However, due to the low vapor pressure of water, adhesives are usually combined with organic solvents of higher vapor pressure to ensure its evaporation. [72]

Similar interfacial morphology of both adhesives in etch-and-rinse approach in the current research may be affected by their compositions as both tested adhesives

(Single Bond Universal and Tetric N-Bond Universal) are water-containing, ethanol-based, and hydroxyethyl methacrylate (HEMA)-containing adhesives. The combination of water and ethanol in both adhesives may also help to dilute the viscous monomers and facilitate its infiltration into dentin.

Further, HEMA may enhance wetting properties and dentin penetration efficacy of adhesives. [47] The presence of HEMA in both adhesives may play a key role in their similar interfacial morphology as observed in this study. This may possibly explain the insignificant differences found in the depth of resin tags of the two adhesives in etch-and-rinse strategy at higher solvent evaporation durations in the present study.

2. Effect of different solvent evaporation protocols on depth of resin tag penetration in self-etch strategy:

As self-etching primer is conservative in its etch pattern, it causes less dissolution of the tooth substance. Studies by **Bishara et al**[65] and **Dos Santos et al** [66] showed that the tag lengths of self-etching primers were shorter than tags with prior phosphoric acid etching. In this study as well, it was confirmed that the depth of penetration in self-etch approach is less than with etch-and-rinse strategy.

The present findings are corroborated by the report of **R.H. Sundfeld et al.** in **2005** which revealed that as self-etching adhesive materials eliminate the need of rinsing the tooth structure, the byproducts of dentin yielded by the low pH of the adhesive system may lead to a limited demineralizing action, restricting penetration of the adhesive system to the most superficial dentin layers. Moreover, the mineral components from the smear layer may neutralize the acidity of these self-etching systems.[64]

In the present study, the difference of mean penetration depth of two adhesives was statistically significant for all solvent evaporation durations in self-etch group. Tetric N-Bond Universal demonstrated significantly greater depth of resin tag formation than Single Bond Universal in all solvent evaporation durations. For evaporation time of 5 sec, the difference of means between two adhesives was significant with a **p-value of 0.012**. For samples exposed to evaporation of 15 sec, the difference of means was statistically significant with a **p-value of 0.006**, while for those exposed to 25 sec evaporation, the difference was significant with a **p-value < 0.0001.**

The interaction depth with dentin depends on the pH of the adhesives, depending on which self-etch adhesives may be classified as follows:[67]

A. Ultra-mild (pH > 2.5, 0.2–0.5 µm interaction depth)

B. Mild (pH ≈ 2; 0.5–1 µm interaction depth)

C. Intermediate (pH, 1–2; 1–2 µm interaction depth)

D. Strong (pH < 1, > 5 µm interaction depth, similar to etching with phosphoric acid).

Tetric N-Bond universal contain low levels of acidic monomer and is therefore categorized as **"mild etching adhesives"** with a pH ≈ 2. On the contrary, Single Bond universal adhesive has a pH of 2.7 and thus, can be categorized as an **"Ultra-mild**

Etching Adhesive”[68]. Thus, findings from the present study can be justified as better penetration of resin tag was observed in self-etch strategy with all solvent evaporation durations (0s, 5s, 15s and 25s) using Tetric N-Bond Universal when compared to Single Bond Universal.

3. Effect of different solvent evaporation protocols on microtensile bond strength in etch-and-rinse and self-etch strategy:

It is evident from the finding of the present study that for both self-etch and etch-and-rinse strategies, the difference of mean micro-tensile bond strength between Single Bond Universal and Tetric N-Bond Universal adhesives was statistically insignificant ($p > 0.05$) for all solvent evaporation durations. Thus, both Single Bond Universal and Tetric N-Bond Universal adhesives performed equally in self etch as well as Etch-and-Rinse strategies in terms of achieving adequate bond strengths.

In the present study, the maximum bond strength in etch-and-rinse strategy for Single Bond Universal **(10.67 MPa)** was achieved at solvent evaporation duration of 5 s. These findings are similar to the results of the study by **Chiba et al. in 2006**[23] who suggested that air drying is essential to obtain adequate dentin bond strengths but increased drying time does not significantly influence bond strength. They also reported that the bond strength of the non-air-dried group was not significantly different from the five second drying time, but prolonged drying was very detrimental to bond strength. They concluded that a five-second air-drying time appeared to be appropriate.

In the control group where no air evaporation was done, the mean micro-tensile strength for Single Bond Universal adhesive in Self-Etch strategy was lesser significantly **(p-value of 0.015)** compared to Etch-and-rinse approach; however, the mean values for Tetric N-Bond Universal adhesive differed insignificantly between two strategies (p=0.806).

This can be justified as large amount of solvent remaining on the interface dilutes the concentration of monomers and separates the polymer cross link creating large physical spaces between the reactive species during the polymerization **(Cho et al. 2004[35]; Cadenaro et al., 2009[39])**, thus ultimately affecting mechanical bond strength of the inter-diffusion zone.

In the present study, the microtensile bond strength of Single Bond Universal was found to be significantly higher **(p-value=0.023)** in Etch-and-Rinse strategy as compared to Self-etch approach when the solvent evaporation was performed for 5 s. However, significantly better bond strength for Single Bond Universal was observed when solvent evaporation was performed for 15 s **(p-value= 0.022)** in Self-etch approach compared to Etch-and-Rinse bonding strategy.

These findings suggest that 15 s air-blowing is too strong in etch-and-rinse approach, as it possibly displaces the entire resin from the RDI into the dentinal tubules and resultantly the resin gets too thin. It may be so thin that the entire fluid is oxygen-inhibited and will not properly polymerize. Extensive air-drying may also increase oxygen diffusion deep into the hybrid layer, which may ultimately lead to adhesive phase separation. Similar findings have been observed in a study conducted by **Daneshmehr et al. in 2013** reporting reduced performance of etch-and-rinse adhesives due to overly aggressive air-blowing of adhesive bonding materials, which resultantly decreased their bond strength.[69]

In the present study, as the duration of solvent evaporation was increased to 15 s in etch-and-rinse approach, the bonding agent was deeply infiltrated into the acid-etched dentin leaving negligible adhesive on the resin/dentin interface to form the inter-diffusion zone, accounting for reduced bond strength value at higher evaporation intervals in etch-and-rinse mode. Contrarily, due to limited depth of demineralization in self-etch approach, more amount of adhesive was available to form uniform hybrid layer at the inter-diffusion zone, thus resulting in higher bond strength in self-etch approach.

Another explanation for the above results may be elucidated by findings of **Jayasheel et al. (2017)[68]** who suggested that chemical bonding in Single Bond Universal between 10-MDP and dentin substrate may play an important role in forming stable and durable interfaces by providing acidity for its self-etch capability. The chemical bond occurs via the formation of electrostatic interaction ionic bonds with the calcium ions of the hydroxyapatite crystals forming an insoluble MDP-calcium salt. This chemical bonding provided by 10-MDP molecule can offer excellent mechanical properties combined with high conversion rate of its filled hydrophobic resin, leading to increased bond strengths.

4. Correlation between depth of resin tag penetration and microtensile bond strength:

The depth of penetration of resin tags and microtensile bond strengths for the two adhesives used in this study in both self-etch and etch-and-rinse strategies differed at varying solvent evaporation durations.

In the present study, statistically insignificant differences were observed between correlation of depth of resin tag penetration and microtensile bond strength when solvent evaporation was increased from zero to 25 s, except in the self-etch group of Single Bond Universal adhesive when solvent evaporation duration was increased from 5 s to 15 s.

These findings can be possibly explained by the effects of prolonged air-drying duration on morphology of resin-dentin interdiffusion zone. In the present study, increasing solvent evaporation to prolonged duration of 25 s might physically displace the adhesive further deeper inside the pre-etched dentinal tubules, thus leaving negligible adhesive on the surface for adequate hybrid layer formation. It is known that dental adhesion is an interfacial surface phenomenon, thus the unavailability of bonding agent at resin-dentin interface due to extreme displacement of adhesive into deeper dentinal surface with increased air-drying may have a possible detrimental effect on bond strength of universal adhesives used in the current research.

The enhanced bond strength of Single Bond Universal adhesive in self-etch mode with increasing solvent evaporation up to 15 s in the present study can be explained by its ‘ultra-mild etching’ action with pH of 2.7.[67] Increasing solvent evaporation from 0 s to 15 s may have effects on increasing acid dissolution of inorganic contents and improved penetration of adhesive into the smear layer, thus enhancing infiltration of MDP monomers to form stable calcium salts and allow for better bond strength.

However, **Wagner et al** in **2014** compared the microtensile bond strength and resin penetration into dentine of three universal adhesives applied in two different two etching modes i.e., self-etch or etch-and-rinse and reported that the addition of an etching step did not significantly affect the microtensile bond strength of none of the adhesives, when compared to their self-etch application mode. All pre-etched specimens showed considerably longer resin tags and thicker hybrid layers. Thus, they concluded that application of an etching step prior to universal adhesives improves their dentine penetration but does not affect their bond strength to dentine after 24 h or after thermocycling. Similar bond strength values were observed for the universal adhesives regardless of the two application modes, which makes them reliable for working under different clinical conditions.[41]

Thus, the null hypothesis of this study that there would be no significant difference in the quality of hybrid layer formation and microtensile bond strength in self-etch vs etch-and-rinse strategies after use of two universal adhesives i.e 3M ESPE Single Bond Universal and Ivoclar Vivadent Tetric N-bond Universal with varying evaporation protocol was rejected.

In the present study, the maximum depth of resin tag formation was observed in Etch-and-rinse group with Single Bond Universal adhesive at 25 s of solvent evaporation time. The microtensile bond strength was found to be the highest in self-etch group with Single Bond Universal adhesive at 15 s of solvent evaporation time. Therefore, within the limitations of this study, it can be concluded that Single Bond Universal demonstrated better adhesive performance than Tetric N-Bond Universal in both bonding strategies at varying evaporation protocols.

The findings from the present study are relevant to clinical usage of universal adhesives in the different bonding strategies. It can be concluded that increasing solvent evaporation up to 15 s improves resin infiltration into dentin, promoting better monomer diffusion through smear layer and resultantly enhances bonding performance of universal adhesives in self-etch mode. On the other hand, in etch-and-rinse approach, as the smear layer is completely removed from etched dentin surface, solvent evaporation duration of 5 s may be sufficient to cause adequate resin infiltration into interfibrillar spaces to provide comparable bond strengths.

LIMITATIONS

The findings of the present study have to be seen in light of several possible limitations despite stringent adherence to research protocols:

1. The extracted teeth were stored according to standard guidelines, but they revealed great degree of variations with respect to oral conditions.

2. Exact simulation of the oral conditions was difficult to achieve if not impossible as the present research design was an in-vitro study. Therefore, complex dynamic interactions within intraoral conditions may produce more aggressive responses to the adhesive performance in terms of bond strength, compared with the present in vitro test.

3. The primary limitation to the generalization of these results is the smaller sample size which may have affected the significance of findings relevant to correlation of penetration depth of resin tags and microtensile bond strength.

4. In the present research, the quality of hybrid layer was evaluated as a function of depth of resin tag penetration at the resin-dentin interdiffusion zone. Further research on quantitative aspects of resin tags may establish validity of claims of the present study.

SUMMARY AND CONCLUSION

Universal adhesives are the most recently introduced generation in the realm of dentin bonding agents and have gained popularity amongst clinicians globally due to their versatility of usage in both self-etch and etch-and-rinse modes. Despite availability of numerous universal adhesives commercially, most manufacturers seem to overlook air drying protocol by simply mentioning “gentle air drying”, thus failing to recommend the appropriate solvent evaporation duration after applying the adhesive over tooth substrate to facilitate adequate resin infiltration, which eventually affects bond strength of adhesives.

Prospective studies have reported enhanced clinical bonding performance of universal adhesives with increased solvent evaporation time as it allows for better diffusion of hydrophilic monomers into interfibrillar collagen spaces, thus resulting in superior interdiffusion zone formation. On the contrary, previous research has also revealed detrimental effects of prolonged air drying on bond strength of dental adhesives. Thus, contemporarily there is no consensus regarding appropriate solvent evaporation protocol of universal adhesives in different bonding strategies.

Therefore, the present in-vitro study was carried out to evaluate the quality of hybrid layer formation in self-etch and etch-and-rinse modes after application of two universal adhesives i.e. **Single Bond Universal** and **Tetric N-Bond Universal** in varying evaporation protocols using confocal laser scanning microscope. The relation of hybrid layer quality and microtensile bond strength was also evaluated in the present study.

In the current research, 80 premolars which fulfilled the inclusion criteria were selected. The teeth underwent occlusal grinding to expose mid-cervical dentin and were randomly divided into two groups depending on the bonding strategy used, and further subdivided according to the adhesives applied as follows:

1. Group I: etch-and-rinse

Sub-group I A: etch-and-rinse with Single Bond Universal

Sub-group I B: etch-and-rinse with Tetric N-Bond Universal

2. Group II: self-etch

Sub-group II A: Self-etch with Single Bond Universal

Sub-group II B: Self-etch with Tetric N-Bond Universal

After application of adhesives as per manufacturers recommendations, solvent evaporation was done with oil-free air-water syringe for each group for 0 s, 5 s, 15 s and 25 s following which the tooth specimen was incrementally built up with composite resin and stored in distilled water for 24 hours. All samples were then sectioned longitudinally in mesio-distal direction to obtain two halves each for evaluation of resin tag penetration under confocal microscope and assessment of microtensile bond strength using universal testing machine.

The findings of the present study indicated significant differences in quality of hybrid layer formation and microtensile bond strength amongst the two adhesives in both self-etch and etch-and-rinse modes when applied in varying solvent evaporation protocols. (p-value <0.05)

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

1. Greater depth of resin infiltration with superior quality of hybrid layer was obtained in etch-and-rinse approach when compared to self-etch strategy for both universal adhesives tested.

2. Single Bond Universal adhesive demonstrated better adhesive performance than Tetric N-Bond Universal in terms of resin tag formation and microtensile bond strength in both bonding strategies at varying evaporation protocols.

3. Increasing the solvent evaporation duration to 15 s enhances penetration of adhesive into dentin and improves bond strength of both the adhesives in self-etch strategy.

4. Prolonged air drying of 25 s may produce deleterious effects upon surface characteristics of hybrid layer and the resultant resin tags which seem to be

displaced too far into dentin, which eventually reduced the microtensile bond strength of both the adhesives in self-etch as well as etch-and-rinse bonding strategies.

Taking into consideration the findings of the present study, it can be concluded that under the experimental conditions, a superior quality of hybrid layer with deeper resin tags and highest bond strength is achieved when solvent evaporation is done for 15 s in self-etch mode for Single Bond Universal adhesive. However, further investigations and correlative studies with quality of hybrid layer could provide a conclusive remark on effect of solvent evaporation duration of universal adhesives on their bonding performance.

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TABLES AND GRAPHS

Table 1: Descriptive statistics for maximum depth of penetration of resin tag in each sub-category of two groups corresponding to varying evaporation time

Time	Groups											
	Group I: Etch-and-rinse						**Group II: Self-etch**					
	Sub-groups						Sub-groups					
	Group I A: Single Bond Universal			**Group I B: Tetric N-bond Universal**			**Group II A: Single Bond Universal**			**Group II B: Tetric N-bond Universal**		
	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median
Control	90.73	13.45	92.42	48.21	7.93	48.15	45.97	9.76	44.27	60.64	7.04	64.52
5 Sec	168.71	22.66	164.61	126.77	25.03	131.86	90.54	9.45	88.67	105.61	4.56	106.36
15 Sec	200.14	44.90	184.12	143.80	39.69	161.95	96.81	7.55	98.83	112.61	5.74	111.10
25 Sec	250.91	96.81	202.57	196.13	21.22	192.03	99.28	4.16	99.10	129.40	10.4	131.83

Table 2: Descriptive statistics for micro-tensile bond strength in each sub-category of two groups corresponding to varying evaporation time

Time	Groups											
	Group I: Etch-and-rinse						**Group II: Self-etch**					
	Sub-groups						Sub-groups					
	Group I A: Single Bond Universal			**Group I B: Tetric N-bond Universal**			**Group II A: Single Bond Universal**			**Group II B: Tetric N-bond Universal**		
	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median
Control	4.84	1.43	4.50	3.61	1.70	3.92	2.14	1.33	1.80	3.34	1.69	3.55
5 Sec	10.67	6.08	12.60	5.49	2.74	5.35	2.96	0.93	2.98	4.22	2.12	3.34
15 Sec	7.07	1.65	7.20	10.68	4.13	10.30	11.13	2.75	10.55	8.48	1.43	8.80
25 Sec	6.70	2.36	6.25	8.08	2.53	7.95	8.10	3.24	8.50	5.40	1.65	5.58

Table 3: Comparison of maximum penetration depth between two sub-categories of each group for varying evaporation time

Time	Groups													P-value*
	Group I: Etch-and-rinse						P-value*	**Group II: Self-etch**						
	Sub-groups							Sub-groups						
	Group I A: Single Bond Universal			**Group I B: Tetric N-bond Universal**				**Group II A: Single Bond Universal**			**Group II B: Tetric N-bond Universal**			
	Mean	SD	Median	Mean	SD	Median		Mean	SD	Median	Mean	SD	Median	
Control	90.73	13.45	92.42	48.21	7.93	48.15	< **0.0001**	45.97	9.76	44.27	60.64	7.04	64.52	**0.026**
5 Sec	168.71	22.66	131.86	126.77	25.03	164.61	**0.024**	90.54	9.45	88.67	105.61	4.56	106.36	**0.012**
15 Sec	200.14	44.90	184.12	143.80	39.69	161.95	0.069	96.81	7.55	98.83	112.61	5.74	111.10	**0.006**
25 Sec	250.91	96.81	202.57	196.13	21.22	192.03	0.252	99.28	4.16	99.10	129.40	10.40	131.83	< **0.0001**

*Obtained using t-test for independent samples; Bold p-values indicate statistical significance

Table 4: Comparison of micro-tensile bond strength between two sub-categories of each group at varying evaporation time

Time	Groups													P-value*
	Group I: Etch-and-rinse						P-value*	**Group II: Self-etch**						
	Sub-groups							Sub-groups						
	Group I A: Single Bond Universal			**Group I B: Tetric N-bond Universal**				**Group II A: Single Bond Universal**			**Group II B: Tetric N-bond Universal**			
	Mean	SD	Median	Mean	SD	Median		Mean	SD	Median	Mean	SD	Median	
Control	4.84	1.43	4.50	3.61	1.70	3.92	0.249	2.14	1.33	1.80	3.34	1.69	3.55	0.251
5 Sec	10.67	6.08	12.60	5.49	2.74	5.35	0.121	2.96	.93	2.98	4.22	2.12	3.34	0.256
15 Sec	7.07	1.65	7.20	10.68	4.13	10.30	0.107	11.13	2.75	10.55	8.48	1.43	8.80	0.092
25 Sec	6.70	2.36	6.25	8.08	2.53	7.95	0.401	8.10	3.24	8.50	5.40	1.65	5.58	0.136

*Obtained using t-test for independent samples

Table 5: Comparison of maximum penetration depth for each adhesive between two groups at varying evaporation time

Time	Groups												P-value* (A)	P-value* (B)
	Group I: Etch-and-rinse						**Group II: Self-etch**							
	Sub-groups						Sub-groups							
	Group I A: Single Bond Universal			**Group I B: Tetric N-bond Universal**			**Group II A: Single Bond Universal**			**Group II B: Tetric N-bond Universal**				
	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median		
Control	90.73	13.45	92.42	48.21	7.93	48.15	45.97	9.76	44.27	60.64	7.04	64.52	**< 0.0001**	**0.031**
5 Sec	168.71	22.66	131.86	126.77	25.03	164.61	90.54	9.45	88.67	105.61	4.56	106.36	**< 0.0001**	0.075
15 Sec	200.14	44.90	184.12	143.80	39.69	161.95	96.81	7.55	98.83	112.61	5.74	111.10	**0.001**	0.121
25 Sec	250.91	96.81	202.57	196.13	21.22	192.03	99.28	4.16	99.10	129.40	10.40	131.83	**0.008**	**< 0.0001**

*Obtained using t-test for independent samples; A: 3M ESPE Single Bond Universal; B: Ivoclar Vivadent Tetric N-bond Universal; Bold p-values indicate statistical significance

Table 6: Comparison of micro-tensile bond strength for each adhesive between two groups at varying evaporation time

Time	Groups												P-value* (A)	P-value* (B)
	Group I: Etch-and-rinse						**Group II: Self-etch**							
	Sub-groups						Sub-groups							
	Group I A: Single Bond Universal			**Group I B: Tetric N-bond Universal**			**Group II A: Single Bond Universal**			**Group II B: Tetric N-bond Universal**				
	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median		
Control	4.84	1.43	4.50	3.61	1.70	3.92	2.14	1.33	1.80	3.34	1.69	3.55	**0.015**	0.806
5 Sec	10.67	6.08	12.60	5.49	2.74	5.35	2.96	.93	2.98	4.22	2.12	3.34	**0.023**	0.439
15 Sec	7.07	1.65	7.20	10.68	4.13	10.30	11.13	2.75	10.55	8.48	1.43	8.80	**0.022**	0.293
25 Sec	6.70	2.36	6.25	8.08	2.53	7.95	8.10	3.24	8.50	5.40	1.65	5.58	0.461	0.083

*Obtained using t-test for independent samples; A: 3M ESPE Single Bond Universal; B: Ivoclar Vivadent Tetric N-bond Universal; Bold p-values indicate statistical significance

Table 7: Correlation between maximum penetration depth of adhesive and micro-tensile bond strength for each bonding strategy and adhesive used - Control

Groups **[Bonding strategies]**	**Sub-groups** **[Adhesives]**	**Control**	
		Correlation	P-value
Etch-and-rinse	Single Bond Universal	0.011	0.986
Etch-and-rinse	Tetric N-bond Universal	-0.298	0.627
Self-Etch	Single Bond Universal	-0.184	0.767
Self-Etch	Tetric N-bond Universal	0.124	0.842

Table 8: Correlation between maximum penetration depth of adhesive and micro-tensile bond strength for each bonding strategy and adhesive used for evaporation time of 5 Sec

Group **[Bonding strategies]**	**Sub-groups** **[Adhesives]**	**5 Sec**	
		Correlation	P-value
Etch-and-rinse	Single Bond Universal	-0.385	0.522
Etch-and-rinse	Tetric N-bond Universal	-0.926	**0.024**
Self-Etch	Single Bond Universal	0.689	0.199
Self-Etch	Tetric N-bond Universal	-0.137	0.826

Table 9: Correlation between maximum penetration depth of adhesive and micro-tensile bond strength for each bonding strategy and adhesive used for evaporation time of 15 Sec

Group [Bonding strategies]	Sub-groups [Adhesives]	15 Sec	
		Correlation	P-value
Etch-and-rinse	Single Bond Universal	-0.585	0.301
Etch-and-rinse	Tetric N-bond Universal	-0.541	0.347
Self-Etch	Single Bond Universal	0.871	0.054
Self-Etch	Tetric N-bond Universal	0.764	0.132

Table 10: Correlation between maximum penetration depth of adhesive and micro-tensile bond strength for each bonding strategy and adhesive used for evaporation time of 25 Sec

Group [Bonding strategies]	Sub-groups [Adhesives]	25 Sec	
		Correlation	P-value
Etch-and-rinse	Single Bond Universal	0.666	0.221
Etch-and-rinse	Tetric N-bond Universal	0.014	0.982
Self-Etch	Single Bond Universal	0.026	0.967
Self-Etch	Tetric N-bond Universal	-0.047	0.941

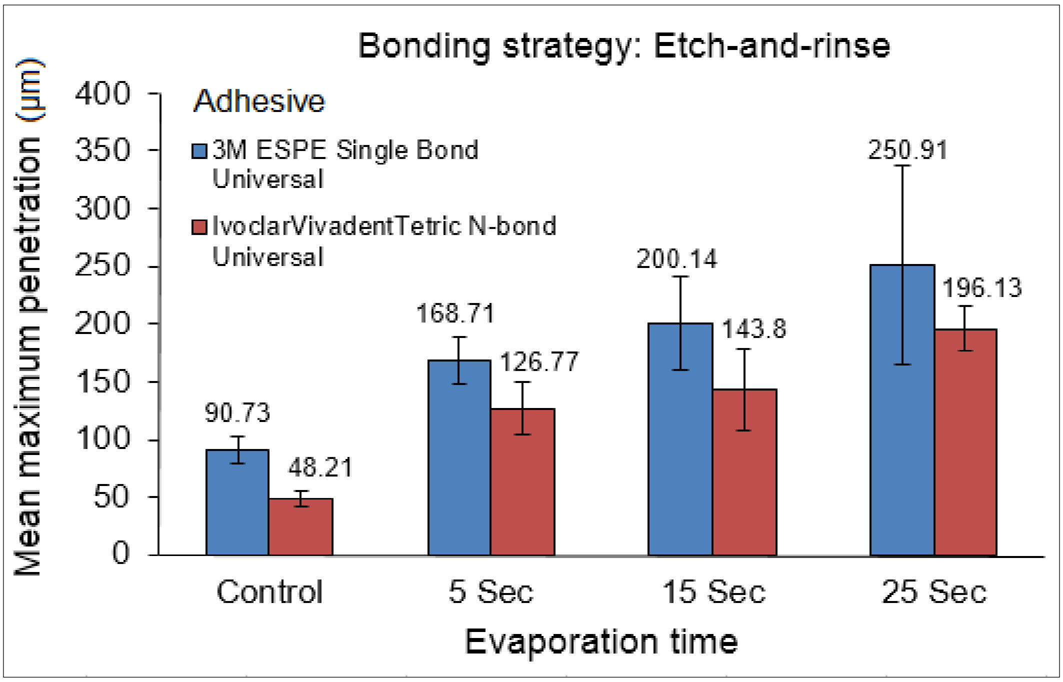

Figure 1: Column chart with error bars showing mean maximum penetration of two adhesives for varying evaporation times: Etch-and-rinse

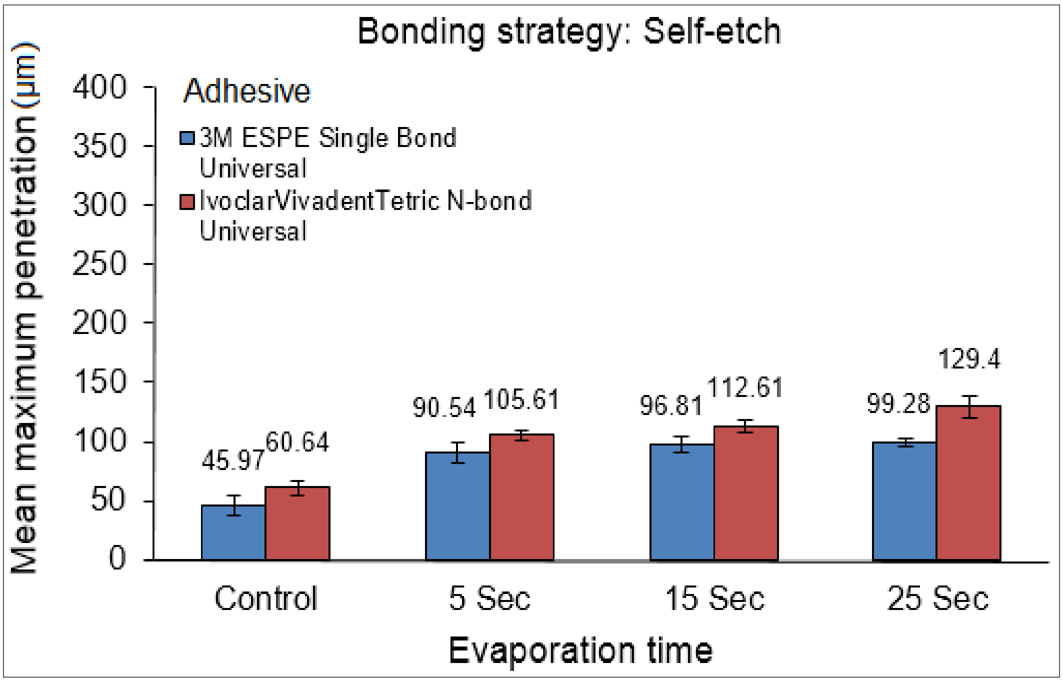

Figure 2: Column chart with error bars showing mean maximum penetration of two adhesives for varying evaporation times: Self-etch

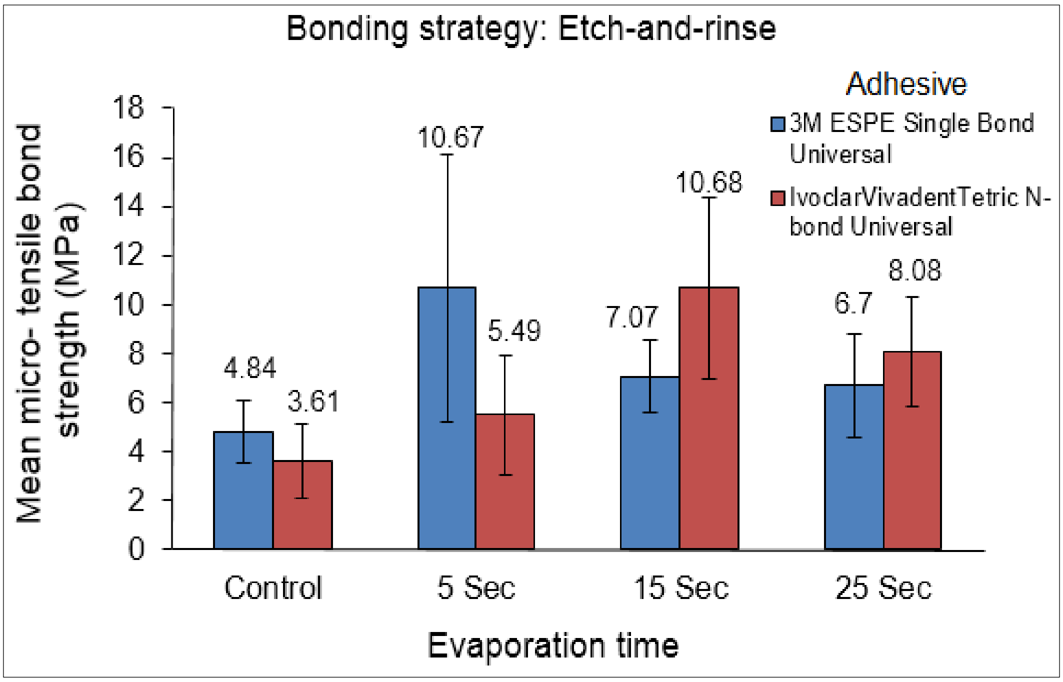

Figure 3: Column chart with error bars showing mean micro-tensile bond strength of two adhesives for varying evaporation times: Etch-and-rinse

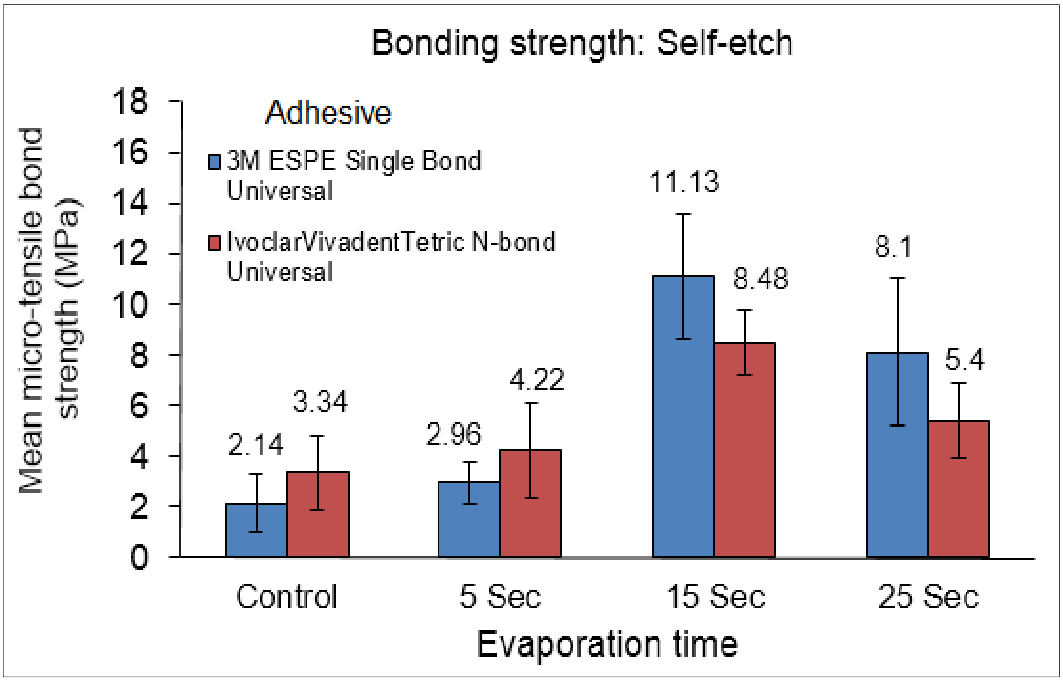

Figure 4: Column chart with error bars showing mean micro-tensile bond strength of two adhesives for varying evaporation times: Self-etch

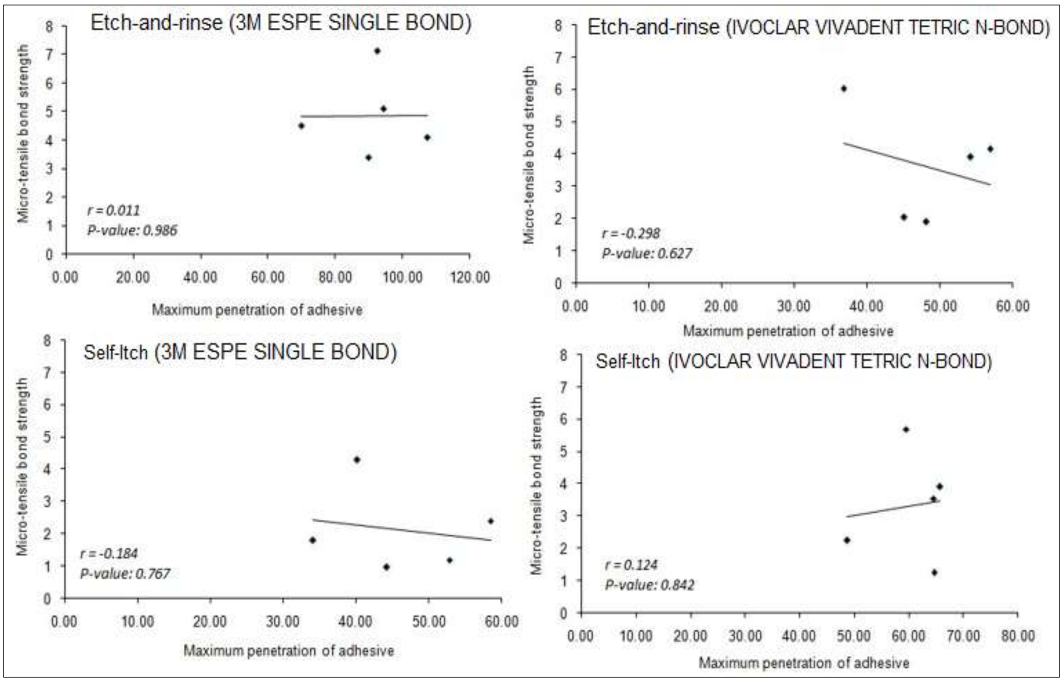

Figure 5: Scatter plots showing relationship of maximum penetration depth and micro-tensile bond strength for two bonding strategies and two adhesives – Control samples

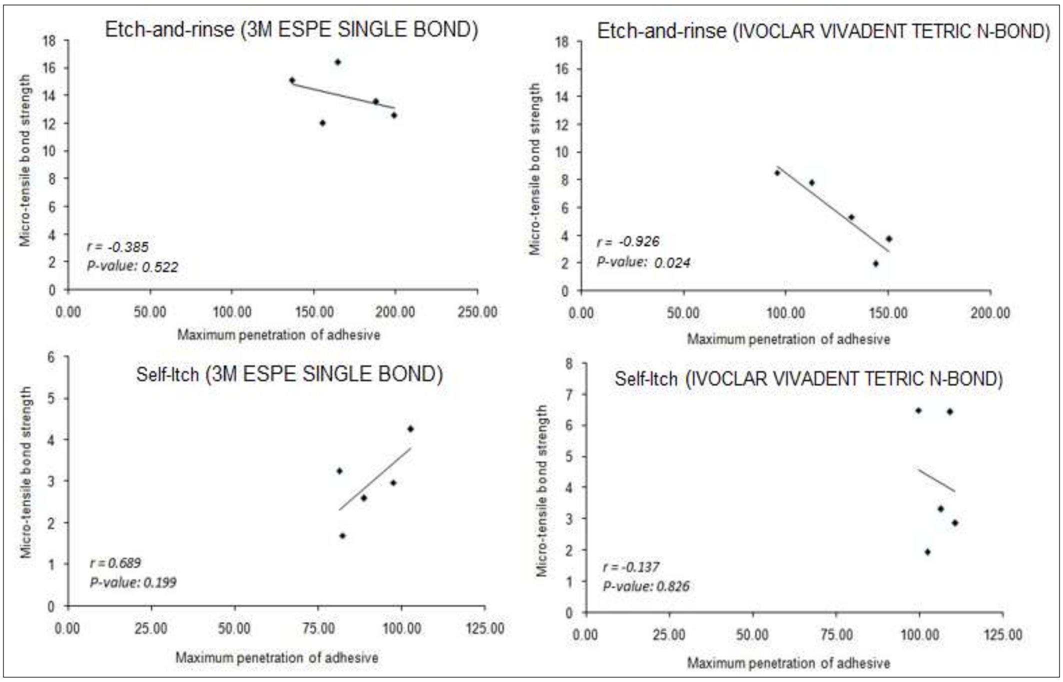

Figure 6: Scatter plots showing relationship of maximum penetration depth and micro-tensile bond strength for two bonding strategies and two adhesives – 5 sec exposure

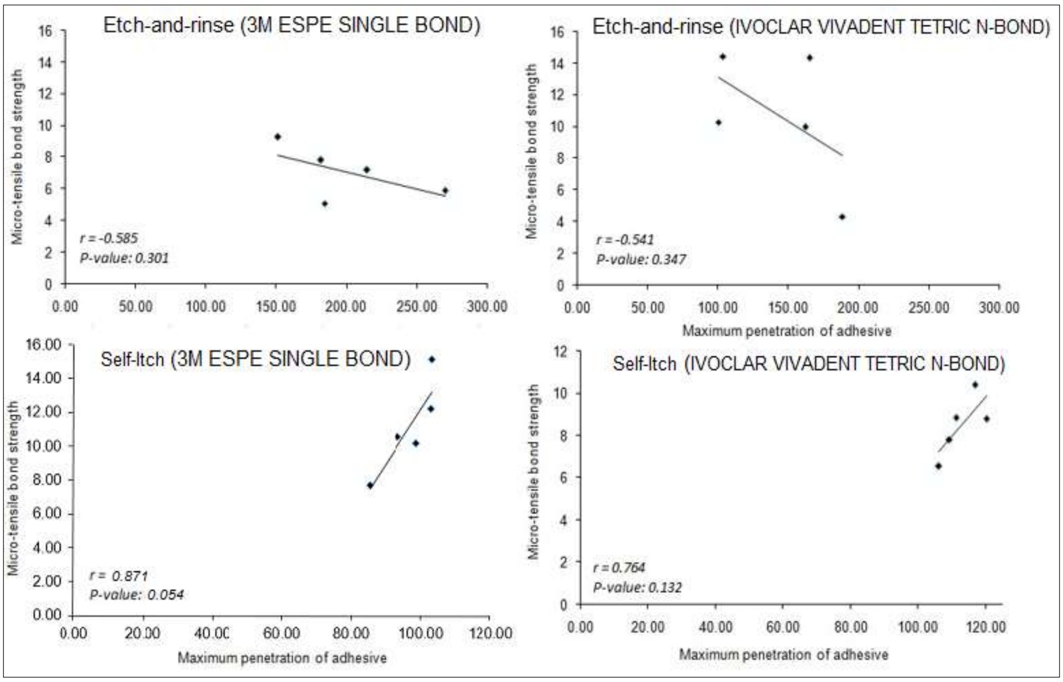

Figure 7: Scatter plots showing relationship of maximum penetration depth and micro-tensile bond strength for two bonding strategies and two adhesives – 15 sec exposure

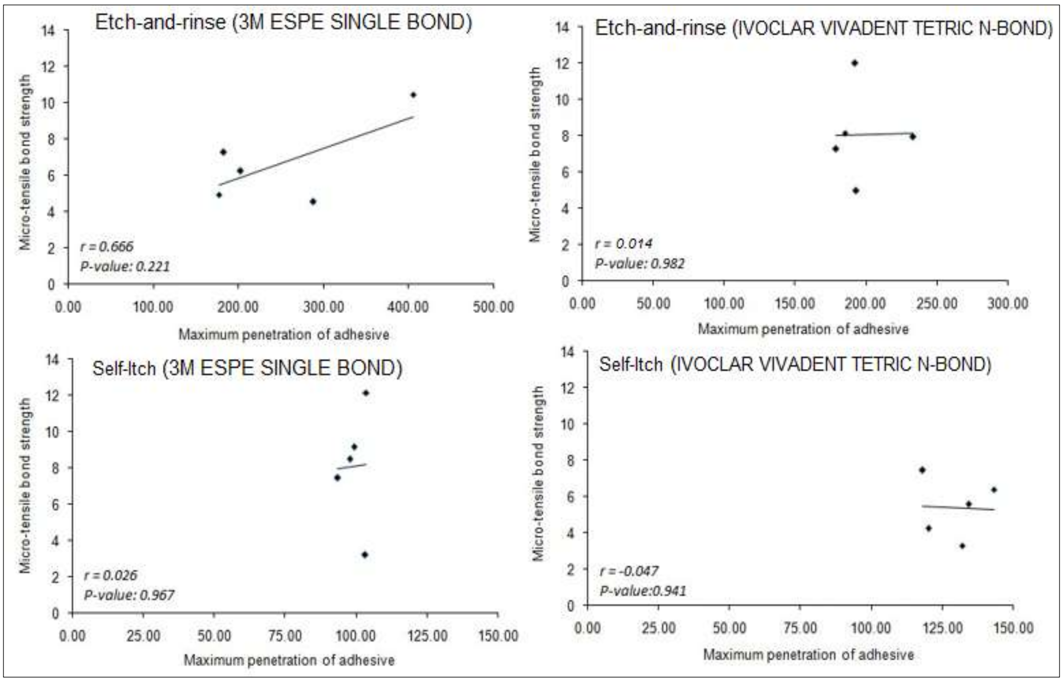

Figure 8: Scatter plots showing relationship of maximum penetration depth and micro-tensile bond strength for two bonding strategies and two adhesives – 25 sec exposure

ANNEXURE I

Depth of penetration of resin tags in Group I A for each section Etch-and-rinse strategy with Single Bond Universal

0 Second	
Sample	Depth of Resin Tag
1	107.274 um
2	89.812 um
3	69.898 um
4	92.424 um
5	94.268 um

5 Seconds	
Sample	Depth of Resin Tag
1	150.416 um
2	143.67 um
3	131.862 um
4	112.358 um
5	95.542 um

15 Seconds	
Sample	Depth of Resin Tag
1	184.116 um
2	151.114 um
3	269.746 um
4	214.394 um
5	181.32 um

25 Seconds	
Sample	Depth of Resin Tag
1	287.82 um
2	404.676 um
3	176.916 um
4	182.578 um
5	202.572 um

ANNEXURE II

Depth of penetration of resin tags in Group I B for each section

Etch-and-rinse strategy with Tetric N-Bond Universal

0 Second	
Sample	Depth of Resin Tag
1	36.782 um
2	54.17 um
3	48.146 um
4	45.074 um
5	56.892 um

5 Seconds	
Sample	Depth of Resin Tag
1	187.91 um
2	164.614 um
3	155.338 um
4	136.7 um
5	198.994 um

15 Seconds	
Sample	Depth of Resin Tag
1	164.928 um
2	188.474 um
3	161.952 um
4	100.204 um
5	103.452 um

25 Seconds	
Sample	Depth of Resin Tag
1	232.654 um
2	192.608 um
3	192.026 um
4	184.898 um
5	178.474 um

ANNEXURE III

Depth of penetration of resin tags in Group II A for each section

Self-etch strategy with Single Bond Universal

0 Second	
Sample	Depth of Resin Tag
1	40.146 um
2	34.106 um
3	44.266 um
4	52.838 um
5	58.486 um

5 Seconds	
Sample	Depth of Resin Tag
1	97.62 um
2	102.8 um
3	82.39 um
4	81.21 um
5	88.666 um

15 Seconds	
Sample	Depth of Resin Tag
1	98.828 um
2	103.33 um
3	85.326 um
4	103.026 um
5	93.514 um

25 Seconds	
Sample	Depth of Resin Tag
1	103.01 um
2	93.302 um
3	103.386 um
4	97.608 um
5	99.098 um

ANNEXURE IV

Depth of penetration of resin tags in Group II B for each section

Self-etch strategy with Tetric N-Bond Universal

0 Second	
Sample	Depth of Resin Tag
1	64.516 um
2	64.632 um
3	65.7 um
4	59.578 um
5	48.79 um

5 Seconds	
Sample	Depth of Resin Tag
1	102.258 um
2	106.364 um
3	110.58 um
4	109.106 um
5	99.74 um

15 Seconds	
Sample	Depth of Resin Tag
1	116.752 um
2	111.104 um
3	120.096 um
4	105.95 um
5	109.164 um

25 Seconds	
Sample	Depth of Resin Tag
1	134.086 um
2	120 um
3	131.826 um
4	143.068 um
5	118.01 um

ANNEXURE V

Micro Tensile bond strength of Resin Tags in Group I A for each section

Etch-and-rinse strategy with Single Bond Universal

0 Second	
Sample	Micro Tensile Bond Strength (MPa)
1	4.09
2	3.38
3	4.50
4	7.14
5	5.10

5 Seconds	
Sample	Micro Tensile Bond Strength (MPa)
1	13.59
2	16.40
3	12.05
4	15.10
5	12.60

15 Seconds	
Sample	Micro Tensile Bond Strength (MPa)
1	5.10
2	9.30
3	5.88
4	7.20
5	7.85

25 Seconds	
Sample	Micro Tensile Bond Strength (MPa)
1	4.56
2	10.46
3	4.95
4	7.30
5	6.25

ANNEXURE VI

Micro Tensile Bond Strength of Resin Tags in Group I B for each section

Etch-and-rinse strategy with Tetric N-Bond Universal

0 Second	
Sample	Micro Tensile Bond Strength (MPa)
1	6.02
2	3.92
3	1.91
4	2.05
5	4.15

5 Seconds	
Sample	Micro Tensile Bond Strength (MPa)
1	3.75
2	1.98
3	5.35
4	7.80
5	8.55

15 Seconds	
Sample	Micro Tensile Bond Strength (MPa)
1	14.32
2	4.35
3	9.97
4	10.30
5	14.45

25 Seconds	
Sample	Micro Tensile Bond Strength (MPa)
1	7.95
2	4.99
3	12.00
4	8.15
5	7.30

ANNEXURE VII

Micro Tensile Bond Strength of Resin Tags in Group II A for each section

Self-etch strategy with Single Bond Universal

0 Second	
Sample	Micro Tensile Bond Strength (MPa)
1	4.31
2	1.80
3	0.99
4	1.20
5	2.42

5 Seconds	
Sample	Micro Tensile Bond Strength (MPa)
1	2.98
2	4.26
3	1.71
4	3.25
5	2.60

15 Seconds	
Sample	Micro Tensile Bond Strength (MPa)
1	10.15
2	15.09
3	7.66
4	12.20
5	10.55

25 Seconds	
Sample	Micro Tensile Bond Strength (MPa)
1	3.21
2	7.46
3	12.16
4	8.50
5	9.15

ANNEXURE VIII

Micro Tensile Bond Strength of resin tags in Group II B for each section

Self-etch strategy with Tetric N-Bond Universal

0 Second	
Sample	Micro Tensile Bond Strength (MPa)
1	3.55
2	1.26
3	3.93
4	5.70
5	2.25

5 Seconds	
Sample	Micro Tensile Bond Strength (MPa)
1	1.95
2	3.34
3	2.88
4	6.45
5	6.50

15 Seconds	
Sample	Micro Tensile Bond Strength (MPa)
1	10.40
2	8.85
3	8.80
4	6.54
5	7.80

25 Seconds	
Sample	Micro Tensile Bond Strength (MPa)
1	5.58
2	4.26
3	3.31
4	6.40
5	7.45