

A Comparative Study of Shear Bond Strength & Resin Tag penetration and Hybrid layer thickness at the Resin Dentine interface using a V & VI Generation Dentine Bonding Agents – An *in vitro* Study

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Abstract: The purpose of this study is to compare the shear bond strength of two dentine bonding agents, a fifth generation Prime & Bond NT (group I) and a six generation Clearfil Liner Bond 2V (group II) & to correlate the shear bond strength to the resin tag penetration & thickness of hybrid layer at the resin dentine interface produced by SEM.

Sixteen sound premolar teeth were divided into two groups of eight each and were sectioned at the CEJ and mounted on PMMA blocks with buccal surface facing upwards. Buccal enamel was removed. After conditioning & application of bonding agents on exposed dentine surface, brass metal rings of height 4mm & width 4mm were positioned over bonded dentine surface. For Group I TPH composite was packed to fill the metal ring, while for Group II Clearfil APX composite and cured. The test specimen were subjected to Universal Testing Machine (UTM) to test the shear bond strength. For SEM study class I cavities were prep so that the floor of the preparation was 0.5mm below the DEJ. Group I & II were etched & bonding agents applied and filled & cured with corresponding composites. Group III, the etched dentine served as a control. The teeth were sectioned buccolingually with diamond discs & gold sputtered for 45sec in an ion sputtering machine & examined under SEM.

The present study concludes that there is no correlation between shear bond strength and depth of resin tag penetration & thickness of hybrid layer formed at the resin dentine interface.

Key Words: Dentine bonding agents, Shear bond strength, Hybrid layer, Scanning electron microscope

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I. Introduction

The advent of adhesive dentistry has caused a dramatic change in restorative procedures. Currently newer bonding systems are coming into clinical use. These adhesive systems have become more & more acceptable in their clinical performance by enhancing the adhesion of resin to dentine. They vary in their direction of use & technique of application.

Most researchers in the scenario of dentine adhesion have accepted that it is essential to create a hybrid layer at the resin dentine interface in order to obtain proper adhesion. Studies have also demonstrated the presence & formation of hybrid layer or resin impregnated layer which is believed to play a major role in dentine adhesion. Further, it was suggested that penetration of bonding monomers into demineralised dentine & subsequent polymerization forms the hybrid layer or resin impregnated layer. ⁽¹⁾

The term "generation" is used as an arbitrary means of differentiating between various levels of improved handling characteristics & clinical performance. Each successive generation of dentine bonding agents has become simpler to use, as the components have been combined, adhesive component normally associated with bonding has been eliminated & application steps reduced, so that the bonding process is faster. Another advantage of newer generation dentine bonding agents is that they can release fluoride. ⁽²⁾

Current developments in dental adhesives involve the total etch technique, the simultaneous etching of enamel & dentine to improve bond strength. The shear bond strength of composite resin to the surface of dentine is actually greater than the inherent strength of dentine itself under well controlled conditions. Bonding to both types of dental structures permit even better reinforcement to the tooth. ⁽³⁾

Most adhesive interface studies have involved SEM demonstration of the penetration of adhesive resin into demineralised dentine surface with subsequent creation of hybrid layer ⁽⁴⁾. To date, however, it is not clear to what extent the adhesive resin would penetrate & polymerize within the hybrid layer with the different dentine

adhesive systems.

II. Aims & Objectives

Aims:

1. To compare the shear bond strength of two dentine bonding systems, fifth generation-Prime & Bond NT (group I) and sixth generation Clearfil Liner Bond 2V (group II).
2. To evaluate the resin tag penetration & thickness of hybrid layer formed using the same bonding systems by SEM.

Objectives:

The objective is to relate the shear bond strength of the 2 dentine bonding agents to the morphology of the resin dentine interface produced by SEM

III. Material And Methods

Materials :

Table -1: The materials used in this study

Bonding System	Composite	Manufacturer
Prime & Bond NT	Spectrum TPH	Dentsply, De Trey Konstanz, Germany.
Clearfil Liner Bond 2V	Clearfil-APX Composite	Kuraray Co, Ltd Osaka, Japan.

Table -II: Components of bonding system

Bonding System	Generation of dentine bonding system	Components
Prime & Bond NT	Fifth generation	Di & Tri methacrylate resin, PENTA (dipenta erythritol pentacrylate monophosphate) Nanofillers, Amorphous silicon dioxide, Photo initiators, Stabilizers, Cetylamine hydrofluoride, Acetone
Clearfil Liner Bond 2V	Sixth generation	Primer 10 methacryloxy decyl dihydrogen phosphate (MDP), Hydroxyethyl methacrylate (HEMA), Hydrophilic dimethacrylate Camphoroquinone, N-N Diethanol p-toluidine, water Bonding Agent MDP, BisGMA, HEMA Hydrophobic dimethacrylate, dL-camphoroquinone, N-N-diethanol -p-toluidine.



Fig-1 : Prime & Bond NT Adhesive & TPH Composite Resin



Fig-2 : Clearfil liner Bond 2V & Clearfil APX composite resin

Methods :

The study was divided into two parts:

Part -I: Evaluation of shear bond strength

Part -II: Evaluation of resin tag penetration & thickness of hybrid layer formation by SEM.

Part - I : Evaluation of shear bond strength

Preparation of Samples

Sixteen sound premolar teeth, extracted for orthodontic purpose were sectioned at the cemento enamel junction, cleaned of debris & stored in distilled water at room temperature. Sectioning was done using a carborundum disc on a micromotor. The sectioned crowns were mounted on PMMA blocks, such that the buccal surface facing upwards were just submerged below a thin PMMA layer. Buccal enamel was removed by slicing the edge of the tooth mounted in PMMA block on a slow speed diamond saw (ISOMET, Buehler IL, USA) about 1.5 - 2mm from the edge of the block. The exposed dentine surface was measured to 4mm x 4mm dentine for bonding purpose. Samples with pulp horn imminently visible were discarded. Samples thus prepared were divided into 2 groups and stored in distilled water at 37°C.

For the study, the dentine surface was conditioned according to the manufacturer's instruction for the bonding system.

For **Group I** 37% Phosphoric acid tooth conditioner gel was applied to the dentine surface for 15 seconds. Then the surface was rinsed with water to remove the conditioner completely & gently blot dried. Prime & Bond NT bonding agent was applied generously onto the conditioned surface and light cured for 10 seconds.

Group II Tooth surface treatment was done by dispensing equal amount of Clearfil Liner Bond 2V Primers A & B and applied onto the dentine surface with a disposable brush tip and left in place for 30 seconds. The excess volatile ingredient was evaporated with mild oil free air stream. The Bond liquid A was then applied to the conditioned surface with a sponge/ disposable brush tip & light cured for 20 seconds.

Brass rings having 4mm internal diameter & 4mm height were prepared. The rings were positioned over the bonded dentine surface & firmly held in position ensuring that the inner diameter of the ring rested only on dentine.

For **Group I** TPH composite was compactly packed against the dentine surface to fill the ring in 2 increments. Each increment was light cured for 60 seconds.

For **Group II** Clearfil APX composite were packed against the dentine surface in 2 increments & light cured for 40 seconds. The light source Hilux 350 curing light was used for the curing experiments. The samples were stored at 37°C in distilled water for 24 hours prior to testing.

Testing :

The above samples were mounted horizontally onto the lower jaw of the Universal Testing Machine (UTM) - (Model 1011 Instron Co, UK) interfaced with a computer. A 25 gauge S.S. wire loop was hooked around the

brass ring and attached to the upper jaw of UTM which was activated to move upwards at a cross head speed of 1mm/min. Load at fracture was recorded in Newtons.

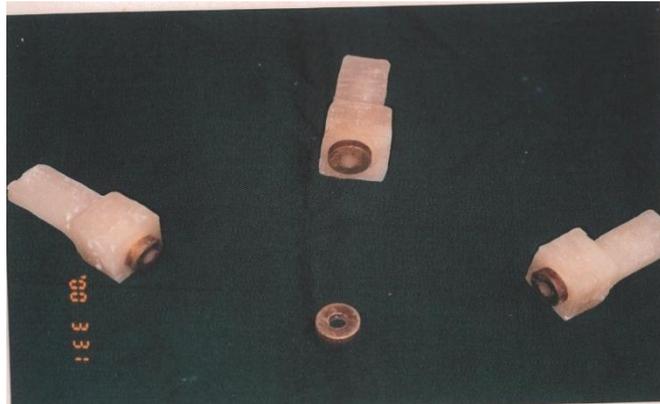


Fig-3 :Brass metal ring & prepared specimens on PMMA blocks



Fig-4 :Slow speed diamond saw (ISOMET)

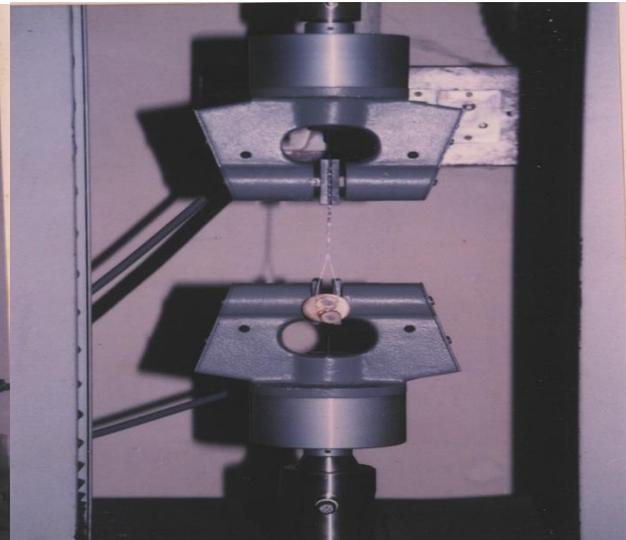
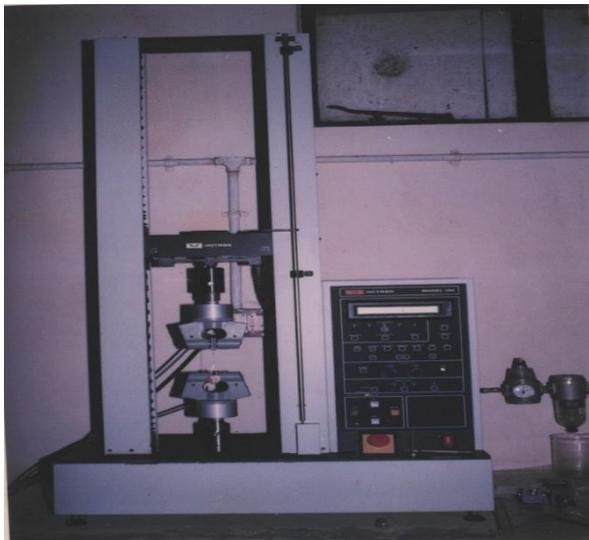


Fig-5 :Universal testing machine (Model 1104 Instron Co) **Fig-6**:Specimen in universal testing machine

Part-II: Evaluation of resin tag penetration & thickness of hybrid layer by SEM

Preparation of samples for SEM

Class I cavities were prepared on recently extracted premolars using diamond bur. The floor of the preparation was placed 0.5mm below DEJ. The class I preparations in Group I&II were etched & bonding agent applied and cured according to the manufacturer's instructions and restored with corresponding composites in incremental technique and cured. Group III which is the etched dentine served as control.

All teeth were stored in saline for 24 hours. Then the teeth were sectioned bucco lingually using diamond discs with frequent cooling. The root portions of all teeth were sliced off. The surfaces were treated with EDTA to remove the smear layer. The specimens were mounted on brass stubs. The stubs were marked I, II & III with a marking pen & gold sputtered for 45 seconds to a thickness of 100°A of gold in an ion sputtering machine (Joel JFL -1200 fine coater). The coated specimens were examined under scanning electron microscope (Figure 11) (Joel JSM 5600 LV SEM) for evaluation of resin tag penetration & hybrid layer formation at $\times 1500$ to $\times 3000$ magnification and hybrid layer thickness and resin tag penetration worked out & photographed.

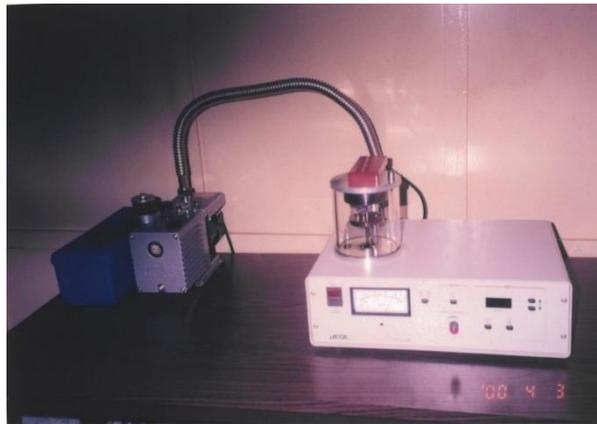


Fig-7 : Ion sputtering machine (Joel JFL-1200 fine coater)

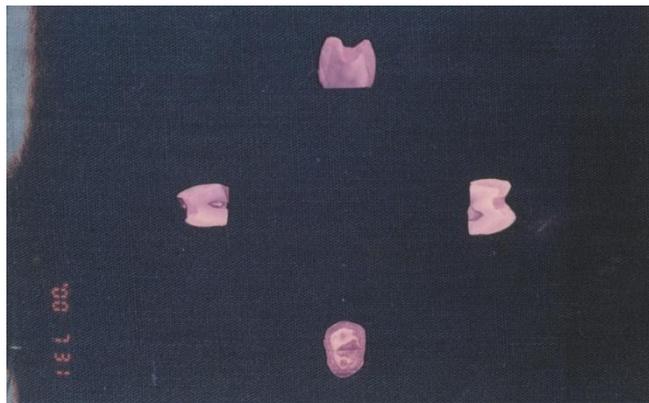


Fig-8 : Gold sputtered specimen



Fig-9: SEM (Joel 5600 LV)

IV. Result & Observations

The load at break & resulting shear bond strength recorded during the shear bond testing using the two bonding systems are listed in Table III & IV and shown graphically. The mean & standard deviation of the assessment variables were computed. The statistical hypotheses formulated were tested statistically by using student's 't' test. Invariably for all the tests the null hypothesis were different for groups having equal mean values. The calculated 't' values were compared with the table values at 14 degrees of freedom. For all statistical computations SPSS computer package was used.

Table – III: Sample – Prime & Bond NT(grp I)

Specimen No.	Load at break (Newton)	Surface areas (mm)	SBS (MPa)
1.	194.6	12.57	15.50
2.	185.7	12.57	14.77
3.	190.3	12.57	15.13
4.	189.6	12.57	15.08
5.	180.9	12.57	14.39
6.	178.8	12.57	14.22
7.	190.5	12.57	15.15
8.	196.3	12.57	15.60

Table – IV: Sample: Clearfil Liner Bond 2V(grp II)

Specimen No.	Load at break (Newton)	Surface areas (mm)	SBS (MPa)
1.	290.4	12.57	23.10
2.	281.4	12.57	22.33
3.	281.9	12.57	22.42
4.	283.6	12.57	22.56
5.	272.9	12.57	21.11
6.	291.4	12.57	23.18
7.	298.1	12.57	23.71
8.	284.67	12.57	22.64

Table – V: Comparison of Mean ± S.D. of assessment variables of group I with group II

Assessment variable	Mean ± S.D		t value	P value
	Group I	Group II		
Load at break	188.3±6.2	285.5±7.66	27.96	P<.0001
Surface area	12.57±0	12.57±0		P>.05
SBS	14.98±0.49	22.63±0.77	23.68	P<.0001

Observations

On comparing Group I with Group II, it is seen that Group II is superior to Group I. In Group II the mean load at break was 1.52 times higher than that of Group I. In other words in Group II the mean load at break was 285.5 compared to 188.3 in Group I. The increased load at break noted happens to be significant statistically at very high level (P<0.0001). Even in this case the SA happened to be same. The mean SBS showed almost two-fold increase in Group II (Mean = 22.63) compared to Group I (Mean = 14.98). Even in this case the statistical test of equality of means happens to be significant (P<0.0001). Thus, group II showed appreciably high level of effectiveness compared to group I.

Fig-10: Comparison of the mean load at break of 2 dentine bonding systems

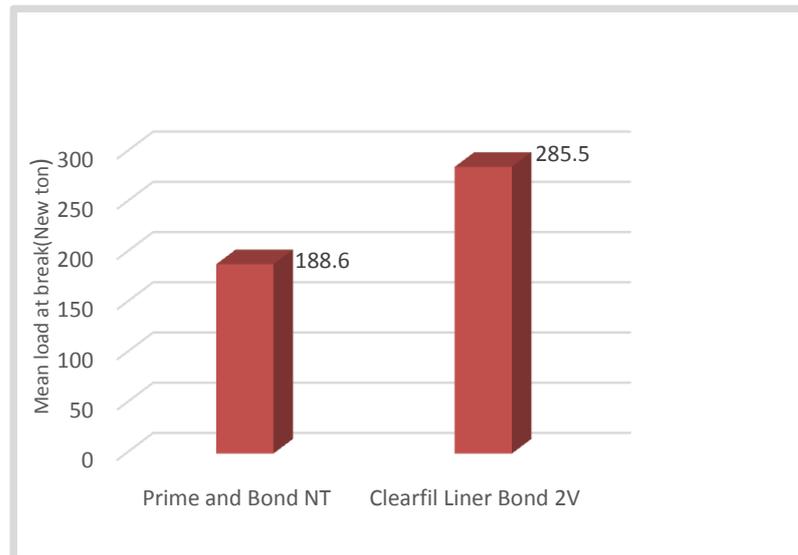
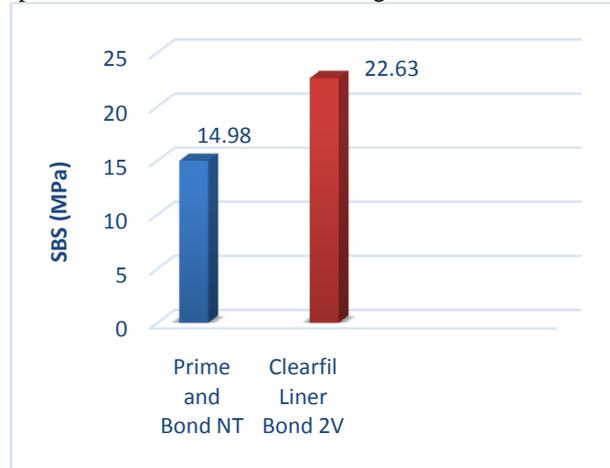


Fig-11 : Comparison of mean shear bond strength of two dentine bonding systems



SEM evaluation of resin dentine interface

Prime & Bond NT (Group I) formed a well infiltrated 3 μ m thick hybrid layer with resin tags extending 55 μ m into the dentinal tubules. In some areas a gap developed between the hybrid layer & polymerized priming resin, alternating with areas of perfect morphological contact.

Clearfil Liner Bond 2V (Group II) formed a hybrid layer of 2 μ m thick with a short resin tags extending 35 μ m into the dentine. The bonding interface is very tight without any gap formation. The top of the hybrid layer is depressed into the dentinal tubule. Group III - Shows dentinal etching using 37% phosphoric acid for 15 sec .

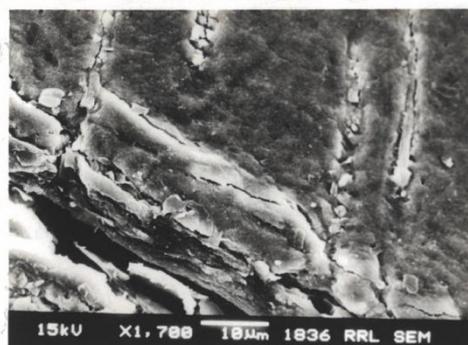


Fig-12 : SEM photograph of resin dentine interface(Prime & bond NT)

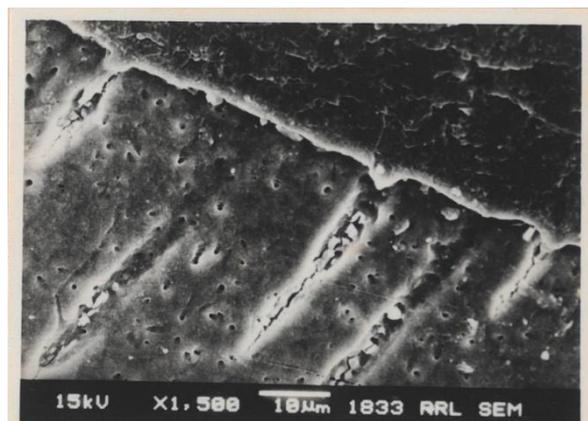


Fig-13 : SEM photograph of resin dentine interface(Clearfil liner bond 2V)

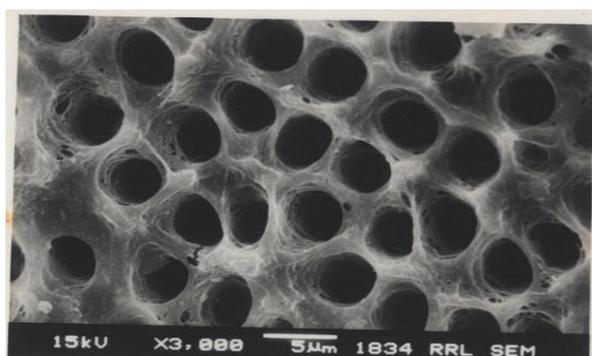


Fig-14 : SEM photograph of etched dentin

V. Discussion

Considerable improvement in dentine adhesion has occurred in recent years with the introduction of hydrophilic primers and "total etch technique". (Fusayama et al, 1979)⁵; (Nakabayashi et al, 1992)⁵. Current developments have focused on simplifying the application of bonding agents by decreasing the time and steps required for placement. As a result the manufacturers have combined the primer & adhesive or the conditioner and primer into a single component. Requirements for an effective dentine adhesive system include the ability to thoroughly infiltrate the collagen network and partially demineralized zones, to co-mingle and encapsulate the collagen and hydroxy apatite crystallites at the front of the demineralised dentine and to produce well polymerized durable hybrid layer (Gwinnett et al, 1996)⁵; (Nakabayashi et al, 1992)⁵. Dentine is a less favourable substrate than enamel for resin bonding, many factors contribute to the difficulty in bonding, including organic content of dentine, variation in its intrinsic composition, the presence of fluid and odontoblastic processes in the tubules, the presence of the smear layer and the inherent wetness of the surface (Swift et al, 1995)⁶

Some investigators have proposed that chemical adhesion is the principal mechanism of bonding to dentine (Buonocore et al, 1956)⁷; (Asmussen & Uno 1992)⁸ & noted the presence of chemical groups in the collagen molecules which might be available for bonding including hydroxyl, carboxyl, amino & amide groups.

Recent invitro investigations have elucidated a successful micromechanical mechanism of attachment by formation of a resin dentine interface. (Erickson 1992)⁹. Formation of this acid resistant resin impregnated hybrid layer seems to depend on the penetrating qualities & surface behaviour of various hydrophilic resin priming agents and also on the condition and permeability of the dentinal surface. Current adhesive systems contain hydrophilic primers that utilize acetone, alcohol or water as solvents. These solvents carry the resin primers into the demineralized dentine by displacing water from the collagen network. It is considered that acetone and alcohol effectively displace water and therefore, are better facilitators of resin primer infiltration into the collagen network compared to water based adhesive systems (Kanca 1992)¹⁰.

Several concepts of bonding mechanism of adhesive resins to dentine have been proposed. One of them involves tag formation in the dentinal tubules of etched dentine (Norden Vail & Brannstrom 1980)¹¹ according to the general concepts developed in polymer reinforcement (CNRS 1974). But mechanical bond with the dentine by the resin tags resulted in poor bond strength. A second bonding mechanism concept is the formation of precipitates on pretreated dentinal substrate on which an adhesive resin may chemically or mechanically bond (Bowen et al, 1982)¹². A third concept consists of a chemical bond to either inorganic (Anbar & Farley 1974)¹¹ and organic components of the substrate (Munksgaard & Asmussen 1984)¹³. Current mechanism involves a

micromechanical interlocking principle (Nakabayashi., 1992)⁵; (Nakabayashi et al, 1982)¹⁴; (Erickson 1989)¹¹; (Inokoshi et al, 1990)¹¹; (Pashley 1990)¹¹; (Harnirattisai et al, 1991)¹¹. An acidic treatment partially demineralises the superficial zone of dentine, facilitating the diffusion of monomers through the subsurface. The polymerization of monomers therein creates a "hybrid layer of resin reinforced dentine (Nakabayakshi et al, 1982)¹⁴; (Wang & Nakabayashi 1991)¹⁴ on which another resinous restoration may be bonded. The SEM evaluation performed supported such a bonding mechanism. The newly formed hybrid layer may be thought of as an admixture of polymer and dentinal components creating a resin - dentine composite.

In this study, Prime & Bond NT is categorized as representative of fifth generation dentine bonding system. Clearfil Liner Bond 2V have been designated as representatives of sixth generation dentine bonding system. Prime& Bond NT, a fifth generation dentine bonding system is a Nanotechnology light cured dental adhesive. It is a self-priming adhesive in which the manufacturer include molecules in the same bottle that are mostly hydrophilic (PENTA) with others having a more hydrophobic behaviour (UDMA) dissolved in organic solvent like acetone. Prime & Bond NT uses 37% Phosphoric acid gel for less than 15 seconds to etch dentine which superficially removes Ca⁺ and PO₄⁻ ions & exposes collagen. It is a two step smear layer removing adhesive. Nanofillers of Prime & Bond NT penetrated the dentinal tubules & infiltrated the microspaces between the collagen fibers within the hybrid layer. Clearfil Liner Bond 2V is a sixth generation dentine bonding agent. It is a dual cured bonding system & consists of a self etching primer & bonding agent. The primer & bonding agent contains well known adhesion monomer 10 methacryloyloxydecamethylene phosphoric acid (MDP) and HEMA. That MDP has high adhesiveness to etched and primed dentine was reported by Chigira et al, (1991)¹⁵; Fortin et al, (1994)¹⁵ and; Prati et al, (1995)¹⁵. These adhesive systems dissolve the smear layer by either incorporating acidic components into pretreatment or conditioning solutions.

In our investigation an attempt was made to relate dentine shear bond strength to the morphology of resin dentine interface produced by SEM. Prime& Bond NT, the acetone based dentine bonding agent produced a mean shear bond strength of 14.98 MPa. SEM showed a 4µm thick hybrid layer & resin tag penetration of 55 µm. The primer contains acetone which acts as a water chaser to carry resin into etched dentine resulting in good adaptation to the dentine surface and high bond strength. Clearfil Liner Bond 2V is a self etching primer which produced a mean shear bond strength of 22.63 MPa. The micrographs showed a thin hybrid layer of 2µm and tag penetration of 35µm. These bonding systems do not employ separate acid conditioning agents instead they are applied directly to the smear layer. Since the smear layer & smear plugs are present, the transdental permeability is greatly reduced and no significant wetness is present on the dentine surface. The initial penetration of these agents into the smear layer is, therefore facilitated because of lack of dilution by surface water. However, as the acidic primer infiltrates through the smear layer / smear plug complex, increasing concentration of water will be present and may begin to dilute the resin concentration. Since only one layer of primer is applied, water in primer begins to be lost by evaporation. The acidity of the primer is rapidly buffered by smear layer, leaving less acidity available to etch the sound dentine. So self etching primers shows a thin hybrid layer formation.

Requirements for an effective dentine adhesive system include the ability to thoroughly infiltrate the collagen network & partially demineralized zone, to co - mingle & encapsulate the collagen & hydroxy apatite crystallites at the front of the demineralized dentine & to provide a well polymerized durable hybrid layer. Fukushima & Horibe (1993)¹⁶ suggested that the bond strength is dependent on the mechanical properties of hybrid layer, rather than on the layer thickness. Nakabayashi & Saimi (1996)¹⁷ reported that a thicker hybridized dentine was not necessary for higher bond strength & confirmed the hypothesis that there was no correlation between thickness of hybridized dentine & bond strength. A thickness of 0.1 µm of hybridized dentine could be sufficient & 1 µm thickness of complete hybridization produced good bonding. Hybridized dentine could resist secondary caries & also acid & proteolytic resistance to protect the intact dentine & living pulp tissue from any stimuli. Burrow et al (1994)¹⁸ found that there were bonding systems that showed high bond strength even though the hybrid layer was very thin. The quality of hybrid layer appeared to be of greater importance for creation of higher bond strength than the thickness of this layer. Hybrid layer would be the major bonding mechanism in superficial dentine, with little contribution from resin tags, while in deep dentine, resin tags would contribute most of the bond strength. Bond strength may also be related more to a uniform resin penetration than to the depth of penetration into demineralized dentine surfaces. Pashley et al (1995)^{19,20} found that the thickness of hybrid layer or depth of resin tag penetration were not important determinants of resulting bond strength, but the most important factors were related to the variation in the porosity of bonding substrate & intrinsic strength of the resin.

The results obtained in this investigation must not be considered as a precise reflection of what may happen in vivo to conservative restorations executed by interposing specific adhesives. While under taking in vitro investigations, one must realise that there are in vivo parameters which are not found in vitro. In this study it has been found that thick hybridized dentine & increased tag penetration are not necessary to produce high bond strength. High bond strength is necessary for better retention in cavities in dentine without mechanical

undercuts & inhibition of gap formation which leads to microleakage. However, further research is required to study the micromorphological relationship of resin dentine interface following total etch technique *in vivo* using different & most recent generation of dentine bonding agents.

VI. Conclusion

The goal & challenge in restorative dentistry is the achievement of consistent adhesion to tooth structure. The use of dentine bonding systems has led to changes in the conventional concepts of operative dentistry, mainly facilitating a more conservative approach to cavity preparation brought about by adhesion of new restorative materials to tooth structure. While bond strength studies are quite rough categorizing tools for evaluating the efficacy of bonding they are, however, good screening tools to assess the same characteristics among different materials.

The objectives of the present study was to relate the shear bond strength of 2 dentine bonding agents, a fifth generation (Prime and Bond NT) & a sixth generation (Clearfil Liner Bond 2V) to the morphology of resin dentine interface revealed by SEM.

1. Prime & Bond NT produced a mean shear bond strength of 14.98 MPa. Photomicrographs showed a 4 μm thick hybrid layer with resin tags penetrating up to 55 μm into the dentine.
2. Clearfil Liner Bond 2V produced a mean shear bond strength of 22.63 MPa. SEM showed a 2 μm thick hybrid layer with short tags extending 35 μm into the dentine. In this study, no correlation was found between bond strength and hybrid layer thickness & resin tag penetration for the adhesive systems to dentine. *In vivo* studies are necessary to determine efficacy & long term clinical performance of the new generation dentine bonding systems.

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