Research Article

JRAD Journal of Research and Advancement in Dentistry

J Res Adv Dent 2020;10:2:305-313.

Evaluation of the Effect of Multiple Applications of two Different Bonding Agents on Bond Strength and Nano leakage: An In Vitro Study

Joel G Varghese[1] Mohan Gundappa[2] Princy Paul[3] Vikhashini PM[4] Sajin Madanan[5] Vineeth Mathew George[6]

[1]Reader, Department of Conservative Dentistry and Endodontics, RVS Dental College & Hospital, Coimbatore, Tamilnadu, India.
[2]Professor and Head, Department of Conservative Dentistry and Endodontics, RVS Dental College & Hospital, Coimbatore, Tamilnadu, India.
[3]Professor, Department of Conservative Dentistry and Endodontics, Azeezia College of Dental Sciences and Research, Meeyannoor, Kerala, India.
[4]Senior Lecturer, Department of Conservative Dentistry and Endodontics, RVS Dental College & Hospital, Coimbatore, Tamilnadu, India.
[5]Senior Lecturer, Department of Conservative Dentistry and Endodontics, RVS Dental College & Hospital, Coimbatore, Tamilnadu, India.
[6]Senior Lecturer, Department of Conservative Dentistry and Endodontics, RVS Dental College & Hospital, Coimbatore, Tamilnadu, India.

ABSTRACT

Background: The present study evaluated the effect of multiple coatings of adhesive resin on dentin by measuring shear bond strength and quality of nano leakage.

Materials and Methods: Resin – dentin bonded specimens were prepared using two totals etch adhesives. Acetone based adhesive (Prime & Bond NT) and t-butanol based adhesive (XP Bond). During bonding, resin application and air evaporation were done 1, 2, 3 times on acid-etched, moist dentin surfaces.

Results: Mean shear bond strength were calculated using student t-test and one-way ANOVA ($p<0.05$; $n=7$). Additionally, nano leakage of silver nitrate was evaluated by scanning electron microscope.

Conclusions: The results indicated that multiple coats of adhesive application increase the bond strength and decrease the nano leakage.

Keywords: acetone, t-butanol.

INTRODUCTION

The advent of resin composite restorative materials has brought about a revolution in the field of restorative dentistry. Resin composite material, with all its advantages, also poses a challenge to the restorative clinician to find a perfect bonding system and technique.

The adhesion of composite materials to enamel has become easy and reliable technique in restorative treatment which was done routinely. But bonding to dentin is most challenging, since the dentine surface is a heterogeneous vital substrate with a low surface energy and outward movement of dentinal fluid due to positive pulpal pressure[1]. Process of adhesion to dentin by hybridization was given by Nakabayashi et al. Adhesive systems bond to dentin by demineralizing dentin with acidic conditioners followed by infiltration of monomers into the space previously occupied by mineral crystallites forming a hybrid layer[2].

The overall bond strength of resin composite to dentin can be regarded as a summation of the individual bond strengths provided by intratubular

Received: Nov. 2, 2019**: Accepted**: Dec. 19, 2019
*__Correspondence Dr. Joel G Varghese.__
Department of Conservative Dentistry and Endodontics, RVS Dental College & Hospital, Coimbatore, Tamilnadu, India.
Email: joelgkalapura78@icloud.com

pISSN 2278-0076
eISSN 2321-9270

penetration (resin tag formation) and penetration into partially demineralized dentin (hybrid layer). Hybrid layer formation would be the major bonding mechanism in superficial dentin with little contribution from resin tags (as there are few tubules available in superficial dentin to form tags) [3].

In vitro shear bond strength testing is commonly used to quantitavely analyze and rank the performance of adhesive systems on enamel and dentin. It has been proved effective for evaluation and comparison of different adhesive systems and restorative materials[4]. All current adhesive systems are designed to be hydrophilic and contain resin monomers dissolved in acetone or alcohol. Such solvents aid in displacing the remaining water as well as carrying the polymerizable monomers into the opened dentin tubules and through the nanospaces of the collagen web.

The disadvantage of these solvents is their high volatility, so such solutions should be immediately used. Acetone is highly volatile and can evaporate from the dentinal surfaces very rapidly. This volatility presents some unusual monomer diffusion problems which will affect the diffusivity of monomers into the dentin and produce reduced permeability of resin into dentin, so that more than one coat of adhesive application is mandatory. The hypothesis to be tested here is that multiple coating of the adhesives might increase the shear bond strength and decrease the nanoleakage.

Hence, the aim of this study was to evaluate the influence of multiple adhesive coating of two total etch systems acetone based adhesive (Prime and Bond NT) and t-butanol based adhesive (XP Bond) on Shear bond strength & Nanoleakage.

MATERIALS AND METHODS

Forty eight caries free human mandibular permanent molars were used for the study. Occlusal third of the crown was removed with a diamond disc to expose flat dentin surfaces. The teeth were then mounted in auto- curing acrylic resin using a custom made metal jig (six teeth for scanning electron microscopic evaluation were not mounted at this stage). The exposed dentin surfaces were abraded with a diamond point in an air-rotor hand piece to simulate a bur cut dentin surface.The teeth were randomly divided into two groups according to the bonding agent used (n=24). The bonding agents used for the study were 2 total etch systems acetone based adhesive (Prime & Bond NT) and t-butanol based adhesive (XP Bond). The teeth were subdivided into three subgroups each (n=8) depending on the number of adhesive coats.

Group Ia: One coat of acetone based adhesive (Prime & Bond NT)
Group Ib: Two coats of acetone based adhesive (Prime & Bond NT)
Group Ic : Three coats acetone based adhesive (Prime & Bond NT)
Group IIa: One coat of t-butanol based adhesive (XP Bond)
Group IIb: Two coats of t-butanol based adhesive (XP Bond)
Group IIc: Three coats of t-butanol based adhesive (XP Bond)
Group I a (n=8): One coat of acetone based adhesive

(Prime & Bond NT)

The dentin surfaces were etched for 15 seconds using 37% phosphoric acid etchant gel; rinsed for 10 seconds and blot dried with a cotton pellet. Using the applicator tip, apply ***one coat*** of acetone based (Prime & Bond NT) adhesive to the tooth surface. The surface should remain wet for 20 sec. Excess solvent was removed by gentle air drying for 5 second and light cured for 10 sec.

Group I b (n=8): Two coats of acetone based adhesive

(Prime & Bond NT)

The dentin surfaces were etched for 15 seconds using 37% phosphoric acid etchant gel; rinsed for 10 seconds and blot dried with a cotton pellet. Using the applicator tip, apply ***two coats*** of acetone based (Prime & Bond NT) adhesive to the tooth surface. The surface should remain wet for 20 sec. Excess solvent was removed by gentle air drying for 5 second and light cured for 10 sec.

Group I c: Three coats of acetone based adhesive

(Prime & Bond NT)

The dentin surfaces were etched for 15 seconds using 37% phosphoric acid etchant gel; rinsed for

10 seconds and blot dried with a cotton pellet. Using the applicator tip, apply ***three coats*** of acetone based (Prime & Bond NT) adhesive to the tooth surface. The surface should remain wet for 20 sec. Excess solvent was removed by gentle air drying for 5 second and light cured for 10 sec.

Group II a (n=8): One coat of t-butanol based adhesive

(XP Bond)

The dentin surfaces were etched for 15 seconds using 37% phosphoric acid etchant gel; rinsed for 10 seconds and blot dried with a cotton pellet. Using the applicator tip, apply ***one coat*** of t-butanol based (XP Bond) adhesive to the tooth surface. The surface should remain wet for 20 sec. Excess solvent was removed by gentle air drying for 5 second and light cured for 10 sec.

Group II b (n=8): Two coats of t-butanol based adhesive

(XP Bond)

The dentin surfaces were etched for 15 seconds using 37% phosphoric acid etchant gel; rinsed for 10 seconds and blot dried with a cotton pellet. Using the applicator tip, apply ***two coats*** of t-butanol based (XP Bond) adhesive to the tooth surface. The surface should remain wet for 20 sec. Excess solvent was removed by gentle air drying for 5 second and light cured for 10 sec.

Group II c (n=8): Three coats of t-butanol based adhesive

(XP Bond)

The dentin surfaces were etched for 15 seconds using 37% phosphoric acid etchant gel; rinsed for 10 seconds and blot dried with a cotton pellet. Using the applicator tip, apply ***three coats*** of t-butanol (XP Bond) adhesive to the tooth surface. The surface should remain wet for 20 sec. Excess solvent was removed by gentle air drying for 5 second and light cured for 10 sec.

Composite resin build-up:

Following bonding agent application, composite resin build-up was done for all tooth samples using a polymeric cylindrical mould with 6mm diameter and 5mm height as a matrix. The composite was placed in five one mm increments and each increment light cured for 20 seconds.

Shear bond strength testing:

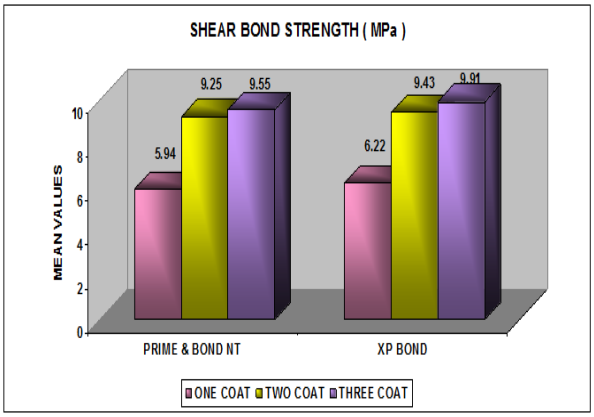
Resin mounted and prepared specimens were stored in distilled water for 24 hours. 7 specimens from each group were mounted in an Instron testing machine and subjected to shear bond strength testing at a cross head speed of 1 mm / minute. Shear bond strengths in MPa were calculated by dividing the maximum force that induced failure by the bonded area.

SEM evaluation:

- Remaining 1 teeth of each group were embedded in self-curing acrylic resin, sectioned occluso-gingivally perpendicular to the resin-dentin interface to obtain 1mm thick sections using a hard tissue microtome.
- Sectioned specimens were coated with nail varnish 1mm away from the bonded interface. The sectioned samples were immersed in 50% (w/v) silver nitrate solution in total darkness for 24 hours, rinsed in running water for 5 minutes; followed by immersion in a photodeveloping solution for 8 hours under a fluorescent light.
- Sections were rinsed under running water for 5 minutes.
- Specimens were then observed under the scanning electron microscope to check for the presence of nanoleakage (2000 X)

Statistical analysis:

Data were analyzed by using student t-test and one-way ANOVA with SPSS version for windows to assess the significance of the group statistics. Post Hoc tests with Tukey HSD, were calculated at $p<0.05$ level, for multiple comparisons between the groups.



Graph 1: Comparison of shear bond strength among all the groups.

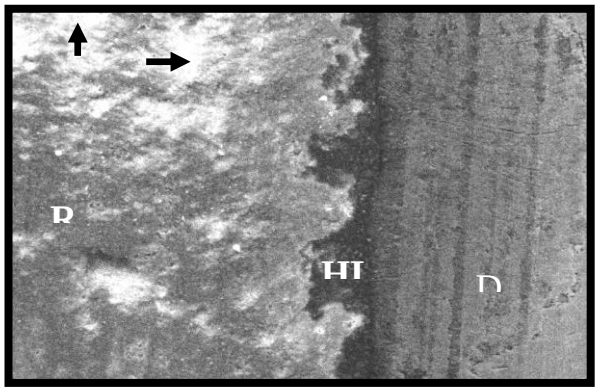


Fig 1: SEM shows heavy silver deposits along resin substrate and hybrid layer.

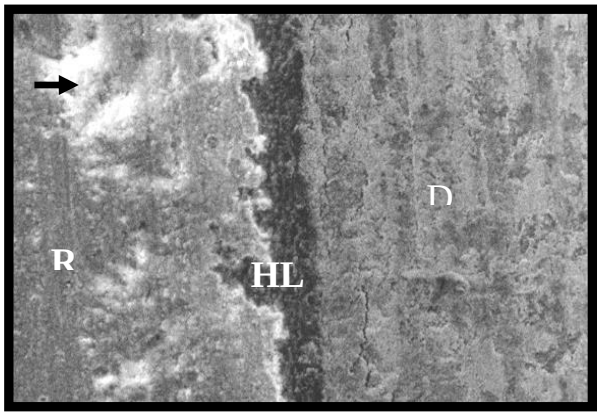


Fig 2: SEM shows silver deposits in some layers of hybrid layer and resin substrate.

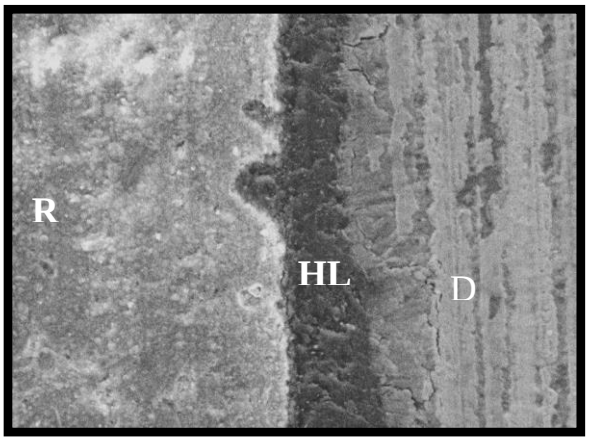


Fig 3: SEM shows no silver deposits.

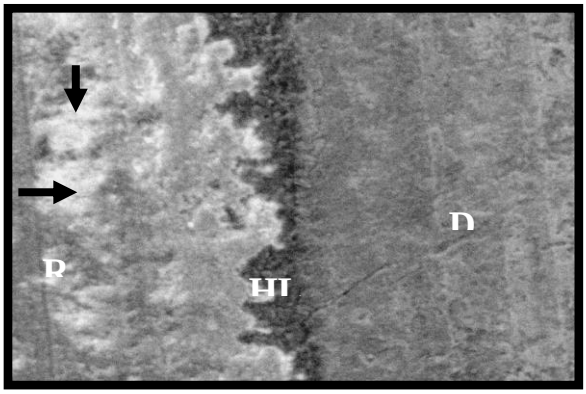


Fig 4: SEM shows heavy silver deposits along resin substrate and hybrid layer.

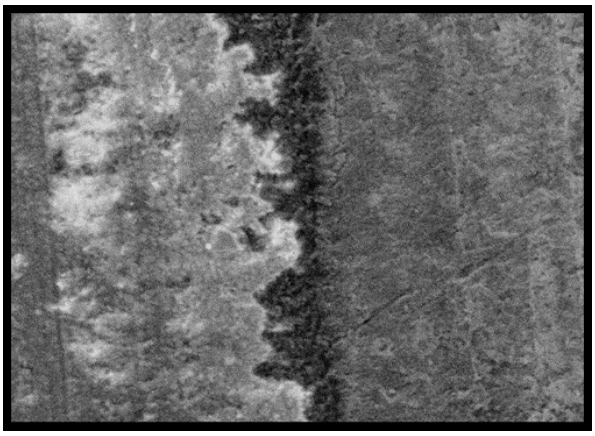


Fig 5: SEM shows silver deposits in some layers of hybrid layer and resin substrate,

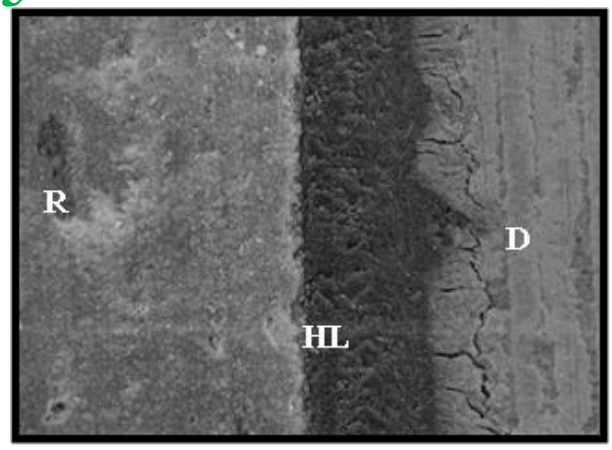


Fig 6: SEM shows no silver deposits

RESULTS

Graph for shear bond testing and SEM pictures for nanolekage evaluation shows that

In Group I- Acetone based adhesive (Prime & Bond NT)

Comparing the shear bond strength between group Ia, Ib and Ic: Ic showed significantly higher bond strength ($P<0.05$). No significant difference between Ia & Ib, and Ib & Ic ($P>0.05$).

In Group II- t-butanol based adhesive (XP Bond)

Comparing the shear bond strength between group IIa, IIb and IIc: IIc showed significantly higher bond strength ($P<0.05$). No significant difference between IIb & IIc ($P>0.05$). Comparing IIa & IIb, IIb showed higher significant difference ($P<0.05$).

Group Ia: One coat prime & Bond NT (fig1) & Group IIa: One coat XP Bond (fig4)

Nanoleakage observations shows heavy silver deposits along the entire adhesive layer as well as the hybrid layer.

Group Ib: Two coats of Prime& Bond NT (fig 2) & Group IIb: Two coats of XP Bond (fig 5)

Silver deposits were present in some areas of the hybrid layer and resin substrate. Silver deposit was less compared to group Ia & group IIa.

Group Ic: Three coats of Prime& Bond NT (fig 3) &Group IIc: Three coats of XP Bond (fig 6) Silver deposits were generally absent

DISCUSSION

Dentin bonding is a combination of micromechanical (tubular penetration & intertubular penetration), chemical (hybrid layer) and hydrodynamic. In short can be described as "A Hydrodynamic Micromechanical bonding"[5]. The approach involves the penetration of resins into partially demineralised dentin and their polymerization in situ.

The presence of a smear layer and it's quality is an important determinant in the bonding of adhesives to dentin. Acid etching removes the smear layer, opens the dentinal tubules, increases dentinal permeability and decalcifies the intertubular and peritubular dentin. Acid etching of dentin removes the mineral phase and increase the porosities of these tissues enormously. In superficial dentin, the surface of dentin changes from one in which only 1% of surface area is porous (resulting from the presence of dentinal tubules) before etching, to a condition in which 13.4% of the surface area consists of water filled tubules that can serve as avenues for infiltration of suitable monomers. Additionally the remaining 86.6% of the surfaces consists of demineralized intertubular dentin that has spaces around every collagen fibril. Thus acid etching converts a solid surface to a porous, non solid surface[6]. Bond strengths of adhesives bonded to dentin prepared with different grits of silicon carbide paper were different and also higher than those bonded to surfaces with diamond or tungsten carbide bur created smear layers. In the present study, smear layer was created by abrading the dentin surfaces with a diamond point to simulate a smear layer which is clinically a more relevant substrate.

Kanca (1992) introduced the concept of wet bonding to dentin after acid etching. He demonstrated that bonding to moist dentin improves bond strength. The collagen structure of moist dentin remains open due to the ability of water to keep interfibrillar spaces from collapsing (pashley & others, 1993), and it facilitates resin infiltration (Jacobsen & Soderholm, 1995). A high quality hybrid layer requires optimal infiltration of adhesive monomers into the demineralized dentin surface and proper polymerization[7]. After the introduction of the concept of wet bonding dentin desiccation is no longer indicated. The moist

bonding technique uses a monomer mixture with hydrophilic groups for the wetting of and penetrating into exposed dentin collagen fibrils. The bonding systems are dissolved in a water chasing solvent for the exchange of water inside the exposed collagen matrix with monomer. This zone is then capable of forming a well polymerized cross-linked polymer network. In the present study a moist bonding protocol was followed. Another factor on which adhesion to dentin depends on is the diffusibilty of the monomer into dentin. The resin monomers are essentially carried by organic solvents[8]. These solvents diffuse through the water channels carrying along with it the monomers. The solvents raise the vapour pressure of the water and cause some of it to become volatile. In addition, they reduce the surface tension of water, allowing spreading of the solvent-monomer mixture along the surfaces coated by water. The solvent thus chases the water present. The remaining solvent present is dried leaving a layer of the primer monomers on the dentinal surface. Thus the diffusibility of the monomers into dentin depends on their solubility in the solvent, the affinity of the monomer for the substrate and the time allowed for penetration.

Most single bottle adhesive systems are used in two layers or coats. The first coating serves to wet the moist surface and begins substituting solvated adhesive comonomers for water in the interfibrillar spaces. Water may diffuse from the demineralized dentin and tubules so rapidly that comonomers undergo phase changes. The second application of adhesive removes or resolubilizes resin globules and is thought to remove more water.[9]

Studies done by Eliades et (2001) showed increase bond strength by the techniques of applying multiple layers and curing successive layers using a self etching primer system.[10] When using multiple coatings of adhesives, there are several techniques that can be employed. If the solvent is evaporated between each coat, the comonomers that exists after each coating should increase, thereby facilitating comonomer infiltration with minimal increase in the thickness of the adhesive layer. These solvated adhesives only contain 25 to 30 wt% comonomers and 70 to 85% solvents (preliminary gravimetric analysis). The presence of the solvent facilitates wetting and spreading of the solvated comonomers and may assist in preserving the width of interfibrillar spaces during infiltration. This may facilitate inward diffusion of the comonomers into the matrix. When most of the solvent is evaporated, the concentration of the adhesives must increase dramatically, although the thickness of the layer may be extremely thin. The cumulative concentration of these constituents must rise in the adhesive layer with each application. Although the thickness of the resin layer increases enough to prevent oxygen inhibition of its entire thickness, it is believed that the final adhesive layer has an improved quality (ie, better cross linking, improved interpenetration of polymer chains, improved percent conversion).

Ito et al has speculated that the bond strength reached a plateau following sequential coatings when a limiting critical variable had been reached (eg, film thickness, cross-linking density, concentration of photoinitiators, accelerators). When each successive layer is light cured, the adhesive layer becomes thicker without changing the quality of hybrid layer. The improved bond strengths may have been due to mechanical rather than chemical factors. After the first layer was cured, it would be unlikely that there could be any more resin infiltration into the matrix. However, it might be possible to improve the quality of each layer of adhesive. That is, since each layer was polymerized, only the oxygen-inhibited portion of the layer would be available to mix with the newly applied layer. In the present study adhesive was applied without light curing each layer.

Reis et al (2003) investigated the microtensile bond strength of ethanol/water and acetone based one bottle adhesive systems to enamel and dentin in the presence or absence of their respective solvents. They came a conclusion that there were no statistical significant difference in mean bond strength among groups restored with or without solvent for enamel. However, a significant reduction in bond strength was observed when both one-bottle adhesive systems were applied without solvent to dentin[11].

Two bonding agents with two different solvents namely acetone based adhesive (Prime & Bond NT) and t-butanol based adhesive (XP Bond) were used for the study. Prime & Bond NT contains DI-and Trimethacrylate resins, PENTA (dipentaerythritol penta acrylate monophosphate), amorphous silicon

dioxide, photoinitiators, stabilizers, cetylamide hydrofluoride and acetone. XP Bond contains carboxylic acid modified dimethacrylate (TCB resin), PENTA, urethane dimethacrylate (UDMA), triethyleneglycol dimethacrylate (TEGDMA), 2-hydroxyethylmethacrylate (HEMA), butylated benzenediol (stabilizer), Ethyl-4-dimethylaminobenzoate, camphorquinone, functionalized amorphous silica and t-butanol.

The role of acetone in the bonding solution is (1) acetone lowers the viscosity of the solution and thus, may enhance the penetration of the bonding agent into the demineralized, collagen rich dentin surface; (2) lowers the surface tension of water, acetone has a 'water chasing' effect; and (3) acetone is increasing the vapor pressure of water suggesting that it may enhance the removal of collagen surface water, which could then be exchanged for the acetone and ultimately for the adhesive resin.

In the present study, we used the shear bond test to analyze the bond strength of adhesives. The shear bond test has been used extensively to evaluate the quality of bonds produced by different restorative materials to enamel and dentin. Scanning electron microscopic evaluation of the resin-dentin interface was also carried out to evaluate the quality of nanoleakage.

Comparing the shear bond strength between group Ia, Ib and Ic: Ic showed significantly higher bond strength. No significant difference between Ia & Ib, and Ib & Ic.

Comparing the shear bond strength between group IIa, IIb and IIc: IIc showed significantly higher bond strength. No significant difference between IIb & IIc, Comparing IIa and IIb, IIb showed higher significant difference.

From the results of our study multiple coatings of adhesive application (3 coats) showed higher bond strength. The possible reason might be due to the increased extent of resin impregnation into collagen caused by the consecutive coating method might remove residual water, thereby, improving resin-infiltration and cross-linking of the adhesive comonomers within the hybrid layer. Our study was in accordance with the study done by Hashimoto et al (2004)[12] which evaluated the effect of multiple consecutive coatings of adhesive resin on dentin by measuring both microtensile bond strength and nanoleakage. They concluded that bond strengths increased with each coat and nanoleakage decreased with each coat. Lower bond strength in one coat application in both the groups can be due to the incomplete resin infiltration into the collagen network. This can be correlated with vapor pressure of the solvent present in the adhesive.

Volatility is the ease with which a compound changes to a vapour state from a liquid state. This property depends on the intermolecular forces of attraction present between the molecules which in turn is directly proportional to the size of the molecules. The magnitude of the intermolecular forces of attraction keeps a compound in the different phases like solid, liquid and gaseous phases. In a liquid when the intermolecular forces of attraction is lesser or is weaker, the liquid tends to vaporize or volatilize.

In acetone the predominant intermolecular forces of attraction are the Vanderwalls forces. Acetone has a formula of CH_3-CO-CH_3. The size of acetone being small results in lower intermolecular forces of attraction so acetone tends to be volatile as these low intermolecular forces of attraction are weak and are broken easily.

t-butanol has the formula -CH_3-CH_2-CH_2- CH_2-OH. It has 4 carbon atoms. Apart from the Vanderwalls forces between the 4 carbon atoms, hydrogen bonding present makes the intermolecular forces of attraction stronger thus making them less volatile in comparison to acetone.

The boiling point of the liquid is the temperature at which the complete conversion of the liquid takes place into a gaseous state. The boiling point for acetone is the least as it is very easy to break the intermolecular bonding. Higher boiling points only refer to the stronger intermolecular forces of attraction between the molecules.

Acetone has the least boiling point of 56.5^0C compared to t-butanol 82^0C. Due to the high boiling point it is advantageous in clinical practice by allowing the use of a dappen dish and the increase of the resin content. Both lead to increased adhesive thickness. Sano et al observed and demonstrated a different pattern of leakage along dentinal wall and hybrid layer. It was termed *nanoleakage* as they

were sub-micron spaces within the hybrid layer in the absence of gap formation. It was hypothesized to be due to the disparities that existed between the depth of demineralization and monomer diffusion that permitted tracer penetration. Li et al reported nanoleakage can occur both in hybrid layer and within the adhesive layer. This was speculated to represent areas of increased permeability within a polymerized matrix in which water was incompletely removed during polymerization. This kind of leakage may allow penetration of bacterial products and dentinal fluid along the interface, which may result in hydrolytic break down of either adhesive resin or collagen within the hybrid layer, thereby compromising the stability of resin-dentin bond.[13]

However, this study failed to create a uniform hybrid layer in one and two coats of adhesive application for both acetone based adhesive (prime & Bond NT) and t-butanol based adhesive (XP Bond). In contrast, there was a uniform and high-quality hybrid layer in three coats of adhesive application in both the groups.

Table 1: The physical and chemical properties of the solvents are tabulated below.

	Acetone	**t-butanol**
Mol. Weight	58.08	74.12
Chemical formula	$(CH_3)_2CO$	$CH_3(CH_2)_3OH$
Appearance	Clear colorless Volatile liquid	Colorless liquid
Odour	Fragrant, mint like	Camphor
Solubility	Miscible in all proportions of H_2O	Miscible in H_2O
Sp. Gravity	0.79 @ 20°C	0.78 @ 26°C
pH	No information	No information
% volatiles by volume @ 21°C	100	100
Boiling point	56.5°C/133F	82°C/180F
Melting point	95°C/139F	26°C/79F
Vapour density AIR =1	2	2.6
Vapour pressure mmHg	400 @395°C 104 F	44@26°C 79F
Evaporation rate BU Ac=1	7.7	1.05

A large amount of silver was taken up in the hybrid layers and the resin substrate in one and two coats of both adhesives. Silver uptake is thought to occur in water filled voids (Tay & others 2002). Residual water in the hybrid layer is thought to occur in regions where resin did not infiltrate the collagen network. The amount of silver uptake was reduced following three consecutive coatings for both the adhesive materials (group Ic and IIc). The repeated procedure of adhesive application and subsequent solvent evaporation may promote improved resin infiltration within the exposed collagen fibrils. This speculation was supported by the reduced amount of silver deposition into the hybrid layer as the number of applications increased.

Study done by pashley & others (2002)[7] using TEM revealed the susceptibility of poorly polymerized resin to silver nitrate staining. These results indicate that defects of resin impregnation and imperfect polymerization of the adhesive resin can create water rich zones that permit silver uptake. During wet bonding, residual water in interfibrillar spaces might decrease polymerization of the bonding resin and/or lead to hydrogel formation of HEMA in the bottom half of the hybrid layer. However, the increased extent of resin impregnation into collagen caused by the consecutive coating method might remove residual water, thereby, improving resin- infiltration and cross linking of the adhesive comonomers within the hybrid layer.

CONCLUSION

Multiple coats of adhesive application increases the bond strength and decrease the nanoleakage.In acetone based adhesive (Prime & Bond NT) minimum of three coats of adhesive application is necessary for higher bond strength and less nanoleakage. In t-butanol based (XP Bond) minimum of two coats of adhesive application is necessary for higher bond strength and less nanoleakage.

CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

REFERENCES

1. Alessandra Reis, Alessandro Loguercio Durability of Resin Dentin Interfaces : Effects of Surface Moisture and Adhesive Solvent Component. *Dental Materials 2004;20, 669-676.*

2. Nakabayashi, Saimi Bonding to Intact Dentin Journal of Dental Research 1996; 75, 1706-1715.

3. Bhuvaneswaran Mohan, Deivanayagam Kandaswamy A confocal microscopic evaluation of resin – dentin interface using adhesive systems with three different solvents bonded to dry and moist dentin – An in vitro study. *Quintessence International 2005; 36, 511-520.*

4. Guiherme Carpena, Paula C Shear bond strength of acetone – based one bottle adhesive systems. Braz Dent J 2006;17, 39-43.

5. Choi KK, Condon JR The effects of Adhesive thickness on polymerization contraction stress of composite. Journal of Dental Research 2000;79, 812-817.

6. Byeong – Hoon, Sabine Effects of the acetone content of single solution dentin agents on the adhesive layer thickness and the microtensile bond strength. *Dental Materials 2004;20, 107-115.*

7. Edna Pashley, Kelli A Effects of one versus two applications of an unfilled, all –in – one adhesive on dentine bonding. Journal of Dentistry 2002;30: 83-90.

8. Franklin R.Tay, John Gwinnett Micromorphological spectrum from overdrying to overwetting acid-conditioned dentin in water-free, acetone-based, single-bottle primer/adhesives. Dental Materials 1996;12, 236-244.

9. Henry Lee, Jan Orlowski Effects of Acid Etchants on Dentin. Journal of Dental Research 1973;52, 1228-1233.

10. Eliades G, Vougiouklakis G Heterogeneous distribution of single- bottle adhesive monomers in the resin – dentin interdiffusion zone. *Dental Materials 2001;17, 277-283.*

11. Reis AF, Oliveira MT The effect of organic solvents on one bottle Adhesives bond strength to enamel and dentin. Operative Dentistry 2003;28, 700-706.

12. Hashimoto M, Sano H Effects of multiple adhesive coatings on dentin bonding. Operative Dentistry 2004;29 4, 416-42.

13. Frankenberger R Perdiagao J No-bottle vs multi-bottle dentin adhesives - a microtensile bond strength and morphological study. *Dental Materials 2001;17, 373 – 380.*