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ORIGINAL RESEARCH ARTICLE

EVALUATION OF HYBRID LAYER THICKNESS AND RESIN TAG LENGTH OF SELF-ETCH ADHESIVES WITH DIFFERENT PH USING CONFOCAL LASER SCANNING MICROSCOPE: AN *IN VITRO* STUDY

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Abstract: The aim of this *in vitro* study is to evaluate the hybrid layer thickness and resin tag length of self-etches adhesives with different pH using Confocal Laser Scanning Microscope (CLSM). Total 40 teeth was prepared and divided into four groups. Group-I (Prime and Bond NT), group-II: (Xeno III), group-III: (Xeno V) and group-IV: (Xeno IV). Each group contains 10 teeth. The samples were mounted on Borosilicate cover glass with cover slip. The excitation laser light was generated with a wavelength 514nm. The image was recorded in fluorescent mode and hybrid layer and resin tag length were visualized as green. They were analyzed, and thickness of hybrid layer and length of resin tag were measured by means of Image Browser software (zeiss, Germany) in micrometers. Results were tabulated and mean obtained. Group-I (Prime and Bond NT) showed the maximum values for hybrid layer thickness & resin tag length compared to group-II (Xeno III), group-III (Xeno V) and group-IV (Xeno IV). Among the self-etching primer group-II (Xeno III) with strong pH (<1) gave higher values for hybrid layer thickness and resin tag length. Among the self-etching adhesives, strong self-etch adhesive showed the maximum hybrid layer thickness and resin tag length. Further studies with more parameters must be conducted to evaluate the resin-dentin interface.

Key Words: Prime and Bond NT; Xeno; Hybrid layer; Resin tag; Quartz-Tungsten Halogen light.

INTRODUCTION

Adhesive dentistry has today become the mainstay of restorative dentistry. Patients demand that their teeth be restored not only functionally and anatomically but also aesthetically. Tooth colored restorative materials are therefore, required to replace lost or missing tooth substance using minimally invasive techniques. The lack of mechanical retention with this approach necessitates the need to adhesively bond restorative material to the tooth. The principal means by which adhesion takes place is by an exchange of calcium and phosphates (inorganic material) from enamel and dentin and replacing the same with resins and nanofillers (hybridization). Simultaneously, the dentinal tubules and its branches are filled with the dental adhesive aid in the formation of retentive resin tags. To achieve this, it traditionally requires two steps.[1,2] One process is by removing inorganic hydroxyapatite from enamel and dentin by phosphoric acid and second is by hybridizing them with hydrophilic and hydrophobic resins. This hybridizing phase may be two steps priming and subsequent bonding or one step priming and bonding. The concept of hybridization of dentin is the bench mark of good bonding. The hybrid layer was originally defined by Nakabayashi in 1982 as "the structure formed in dental hard tissues by demineralization of the surface and subsurface, followed by infiltration of monomers and subsequent polymerization" Dental adhesives are broadly classified into two categories. One is Total Etch also known as Etch and Rinse adhesive.[3,4]

Second is Self-etch or Non Rinse adhesive. Total etch adhesives utilize a separate etching of both the enamel and dentin with phosphoric acid. However, recent advances in utilizing acidic polymerizable monomers have enabled the developments of adhesives which etch and prime / bond without the need for a separate etching and rinsing step. Instead of removing the smear layer, as done with the total etch, these self-etching adhesives modify/dissolve the smear layer and then penetrate through it to further demineralize the superficial surface of the dentin and combine with the collagen and the remaining hydroxyapatite to form a hybrid layer. The concept of self-etching adhesives is based on the use of polymerizable acidic monomers that simultaneously conditions and primes dentin and enamel. They involve two step or one step application. The two step application demineralizes and primes tooth surface simultaneously. The adhesive layer is applied as a second step. One- step bonding materials demineralize, prime, and bond in a single application. The clinical requirements for self-etching enamel-dentin adhesives are the same as for adhesives used in combination with the acid etch technique. Discrepancies between the depth of demineralization and the resin monomer infiltration might be avoided by the concurrent demineralization and infiltration of self-etching adhesives.[5,6,7] Also it eliminates the technique sensitivity of keeping the dentin moist. Depending on the etching strengths Van Meerbeek classified self-etching primers into strong self-etch adhesives with a

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pH of 1 or below, intermediary strong self-etch adhesives with a pH less than 2, Mild self-etch adhesive systems with a pH above 2. Common methods used to visualize bonding structures such as hybrid layer and resin tags in the dentin are Micro Raman Spectroscopy, Transmission Electron Microscopy (TEM), Fourier Transform Infrared Spectroscopic Analysis (FTIR), Scanning Electron Microscope (SEM) or combination of these. Confocal Laser Scanning Microscope (CLSM) is being considered here as the laser penetrates into the thin optical sections below the surface of intact specimens offering superior images of the resin dentin interface. The aim of this *invitro* study is to evaluate the hybrid layer thickness and resin tag length of self-etches adhesives with different pH using Confocal Laser Scanning Microscope (CLSM).

Materials and Methods

Study groups

Group-I: Prime and Bond NT
Group-II: Xeno III
Group-III: Xeno V
Group-IV: Xeno IV

Sample preparation

Forty freshly extracted non carious human third molars were selected and stored in normal saline for the study. The collection, storage, sterilization and handling of extracted teeth were followed according to occupational safety and Health Administration (OSHA) and the Centre for Disease Control and Prevention (CDC) recommendations and guidelines. Occlusal surfaces of all the teeth were ground using slow speed diamond disc (Isomet, Buehelor Ltd USA) attached to a micromotor straight hand piece (300 rpm, NSK, JAPAN) with copious water. This produced a flat bonding surface of dentin with an approximate residual dentin thickness of 1.5 to 2.0mm. The exposed dentin surface was then prepared by polishing with 600 grit silicon carbide paper so as to remove enamel remnants. The teeth were then randomly divided into 4 groups of 10 specimens each.[8,9]

Procedure

Group-I dentin surface was etched using 37% phosphoric acid etchant for 15 seconds and then rinsed with distilled water. An absorbent paper piece was then used to blot dry out the excess water that remained after rinsing off the acid conditioner and the dentin was maintained in a moist condition. Bonding agent was dispensed directly onto a fresh applicator tip applied on the conditioned dentin surface for 20 seconds. Air was blown gently to remove excess solvent. This resultant surface should have a uniform, glossy appearance. The surface was light cured for 20 seconds using Quartz – Tungsten Halogen light (QTH) [QHL-75, Dentsply] with wavelength of 450 Mw/cm2.[10,11] The bonded surfaces were restored with 2 mm of resin composite Z350 applied in increments and cured for 40 seconds, to protect the bonding layer. Group-II samples were prepared by a single step application technique. Self-etching primer was mixed as per manufactures instructions and applied on to the prepared 28 dentin surface and left in place for 20 seconds. Adhesive was spread uniformly by a gentle stream of air pressure and light cured for 20 seconds using QTH curing light [QHL-75, Dentsply] with wavelength of 450 Mw/cm2. The bonded surfaces were restored with 2 mm of resin composite Z350 (3M) applied in increments and cured for 40 seconds, to protect the bonding layer.[13,14]

Group-III was prepared by a single step all in one adhesive. Bonding agent was applied to prepared dentin surface and gently agitated for 20 seconds. The solvent was evaporated thoroughly by air blowing until there was no movement of the adhesive and immediately polymerized by light curing for 20 seconds using QTH curing light (QHL – 75, Dentsply) with wavelength of 450Mw/cm2. The bonded surfaces were later restored with 2 mm of resin composite Z350 (3M) applied in increments and cured for 40 seconds to protect the bonding layer. Group-IV involves a single step all in one adhesive. With an applicator tip, the adhesive was applied and the surfaces were left wet for 15 seconds. Later the surfaces were gently scrubbed with an applicator tip for 15 seconds. A second coat of bonding agent was applied for 20seconds. Excess solvent was removed by gentle air blow for 5 seconds. The resultant surface should have a glossy appearance. The bonding agent was cured for 10 seconds by a QTH curing light (QHL – 75, Dentsply) with wavelength of 450Mw/cm2. The bonded surfaces were restored with 2 mm of resin composite Z350 (3M) applied in increments and cured for 40 seconds to protect bonding layer.[15,16]

Fluorescent Labeling of Bonding Agent

The Visualization of the distribution of bonding agents is greatly enhanced by the incorporation of fluorescent labels. In this study Rhodamine B dye (LOBA-CHEMIE Bombay) was used. The dye was mixed with bonding agent before its application.[17]

Sample Preparation for Confocal Microscopy

All the specimens were stored in distilled water. After 24hrs of storage the teeth were longitudinally sectioned using a slow speed diamond disc (ISOMET, Buehler Ltd USA) attached to a contra angle micromotor hand piece (NSK, JAPAN) with copious water supply. The sectioned surface was polished with a 600 grit silicon carbide paper. The dentin-adhesive interfacial region was examined using a Confocal Laser Scanning Microscope (LSM 510 Meta

Confocal Microscope, Zeiss, Germany). The samples were mounted on Borosilicate cover glass with cover slip. The excitation laser light was generated with a wavelength 514nm. The image was recorded in fluorescent mode and hybrid layer and resin tag length were visualized as green. They were analyzed, and thickness of hybrid layer and length of resin tag were measured by means of Image Browser software (zeiss, Germany) in micrometers. Results were tabulated and mean obtained.[18,19]

Statistical analysis

The data was expressed mean and standard deviation. One way ANOVA was used for analysis. Post hoc followed by Dunnet t test applied to find the statistical significant between groups. P vale less than 0.05 ($P<0.05$) considered statistical significant at 95% confidence interval. Statistical Package for Social Sciences (SPSS 2.0 version) used for analysis.[20]

Results

Group-I (Prime and Bond NT) showed the maximum values for hybrid layer thickness & resin tag length compared to group-II (Xeno III), group-III (Xeno V) and group-IV (Xeno IV) and the values were statistically significant. ($P<0.001$). Among the self-etching primer group-II (Xeno III) with strong pH (<1) gave higher values for hybrid layer thickness and resin tag length, than group-III (Xeno V) with intermediary pH (<2) and group-IV (Xeno IV) with mild pH (>2). Group-IV (Xeno IV) with mild pH gave fewer values for hybrid layer thickness and resin tag length among all the self-etching primers tested.

Discussion

Hybrid layer is the resin-infiltrated enamel, dentin, or cementum. The chemical and physical properties of these zones are very different from those of the original tooth structure, because it has been partially demineralized and then infiltrated with resin. The resulting structure is neither resin nor tooth but a hybrid of two. Resin tag is the extension of adhesive resin into open dentinal tubules. The hybrid layer not only promotes good bond strength, but also behaves like an impermeable membrane that can prevent noxious stimuli from invading pulpal tissue through the dentinal tubules. Hybridized dentin reduces the risk of micro leakage, incidence of secondary caries and post-operative sensitivity. Hybridized dentin in a molecular level is a mixture of collagen and resin polymers. It is prepared in the conditioned dentin by the polymerization of resin monomers impregnated in the matrix of demineralized dentin. Mineralized dentin usually does not permit much monomer diffusion into its substance. Therefore, dentin must be suitably conditioned to permit diffusion of monomers, which should have a good affinity for demineralized dentin, into the substrate. Prepared dentinal surfaces are covered with a smear layer that adheres weakly to the underlying intact dentin. Dentin conditioning involves the removal or modification of the smear layer to permit monomer diffusion into the demineralized collagen matrix. Based on the number of application steps, Van Meerbeek (2001) classified adhesives as:

- Etch and rinse adhesives
- Self-etch adhesives
- Glass Ionomer (resin modified) adhesives

In order to overcome the phenomenon of over wetting and over drying Nakabayashi and his colleagues hypothesized that if the bonding step with acidic conditioner to remove smear layer was eliminated, then the bonding procedure would become simpler. This led to the development of self-etching/ self-priming system, where the monomer could penetrate into underlying intact dentin to form both hybridized smear layer and hybridized dentin. These bonding systems do not employ separate acidic conditioning solutions as mentioned earlier. Instead they are applied directly to the smear layer. In addition to simplifying the bonding technique, the elimination of the rinsing and drying step reduces the possibility of over wetting or over drying which can have a negative influence in adhesion. The actual rationale behind these systems is to superficially demineralize dentin and to simultaneously penetrate it to the depth of demineralization with monomers that can be polymerized in situ.

Self-etching primers use non-rinse acidic monomers that simultaneously dissolve/modify the smear layer, demineralize the dentin surface beyond the smear layer and prime dentin as well as the enamel. The adhesive in two-step self-etching adhesives agent is often a solvent- free component. Newer self-etching systems combine the etchant, primer and adhesive in one container. As they etch, they also infiltrate the exposed collagen with hydrophilic monomers, where they copolymerize with the placed adhesive resin to the same depth in the dentin. The result is the formation of a hybridized complex; a hybridized smear layer and hybrid layer which thickness is related to the aggressiveness of the self-etching agent. With these systems the smear layer is a bonding substrate. Moreover, these systems are used under dry bonding conditions. For self-etching adhesives, the ability to remove dentin or enamel smear layer and the formation of an etching pattern on enamel are major requirements for reliable bonding. Therefore, the etching ability of an adhesive plays a major role in comparison and evaluation of self-etching adhesive systems. An established and often used property for the quantification of acidity of adhesives is their pH values.

The pH value of most self-etching primers is between 1.0 and 2.6. The pH value of self- etching adhesives depends on several factors, such as water content, the amount and type of acidic monomer, polarity, di-electric constant, and composition of the co-monomer and solvents. Self-etching adhesives with lower pH values possess greater acidity and therefore produce more pronounced etching patterns on enamel that are comparable to those achieved using phosphoric-acid etching. Van Meerbeek (2001) classified self-etching primers based on their pH into strong, intermediary and mild self-etch adhesives.

- Strong self-etch adhesives with a pH of 1 or below e.g. xeno III.
- Intermediary self-etch adhesives with a pH less than 2 e.g. xeno V.
- Mild self-etch systems with a pH more than 2 e.g. xeno IV.

Common methods used to visualize bonding structures such as hybrid layer and resin tags in dentin are transmission electron microscopy (TEM), Scanning Electron Microscopy (SEM), Fourier Transform Infrared spectroscopic analysis (FTIR) and Micro-Raman Spectroscopy. TEM requires an embedding process. SEM sample preparation technique can result in shrinkage of the bottom half of the hybrid layer which is often poorly infiltrated.

Confocal microscope provides a significant imaging improvement over conventional microscopes. It creates sharper, more detailed 2D images, and allows collection of data in three dimensions. The confocal microscope has an advantage of obtaining laser penetrated thin optical sections below the surface of intact specimens. This eliminates the artifacts that arise with manual sectioning and offers superior images of the resin dentin interface. Another advantage of using confocal microscopy is that out of focus blur is absent from confocal images, giving the capability for direct noninvasive serial optical sectioning of intact and even living specimens. Main advantage of using confocal microscope for evaluating the adhesive layer of different bonding agents is that samples can be kept humid during the examination. Evaluation of the dentin/restorative interface morphology as well as visualization of the distribution of bonding agent components throughout the demineralized dentin can be greatly enhanced by incorporation of dyes into bonding agents. In the present study Group 1 (Prime and Bond NT) showed the maximum values for hybrid layer thickness (5.56μm) and resin tag length (326.16μm) when compared to Group 2 (Xeno III), Group 3 (Xeno V) and Group 4 (Xeno IV) and the values were statistically significant. ($P<0.001$)

Possible reasons could be, in case of Group I (Prime and Bond NT) the specimens were etched with phosphoric acid and the reaction products were rinsed off. This results in complete removal of smear layer and smear plugs from the dentin so that the dentin permeability increases and there is better infiltration of resin, and hence aids in the better penetration of the resin tags and also thick hybrid layer. Another factor could be when a water free acetone-based system such as Prime and Bond NT is applied, the acetone solvent can effectively displace water from the dentin surface and moisten the collagen network. Subsequently, the adhesive resin monomer can completely infiltrate into the sub-fibrillar space of the collagen network and provide excellent micromechanical retention due to affinity between fibrils and resin. Group II, III, and IV showed low values for hybrid layer thickness and length of resin tag formation when compared with etch and rinse adhesive. In self-etch systems, the primer is not rinsed after application but air dried only. The calcium and phosphate ions that were dissolved from hydroxyapatite crystals must have been suspended in the watery solution of the primer. When the water is evaporated during air drying the concentrations of solubilized calcium and phosphate ions within the primer may exceed the solubility constant for a number of calcium phosphate salts. Presumably minerals will then precipitate within the primer. This high concentration of calcium phosphate will tend to limit further dissolution of apatite due to the common ion effects of calcium and phosphates and thereby limit the depth of surface demineralization.

Group 2 (Xeno III) showed the highest values of hybrid layer thickness (4.56μm) and resin tag length (185.77μm) compared to other self-etching adhesives. Strong self-etching primers contain monomers based on phosphoric acid or derivatives with alkyl substitutions. Mozner, Salz and Zimmermann *et al.,* considered acidic phosphates as being more acid than other acidic groups used in self-etching priming agents except for sulfonic acid and that phosphoric acid esters substituted by mono or di-alkyl groups are stronger acids than phosphoric acid itself. Strong self-etch adhesives have a pH of 1 or below, this high acidity results in rather deep demineralization effects. At enamel, the resulting acid etch pattern resembles a phosphoric-acid treatment following an etch and rinse approach

(Pashley and Tay *et al.,* 2001). At dentin, collagen is exposed and nearly all hydroxyapatite is dissolved. Consequently the underlying bonding mechanism of "strong" self-etch adhesives is primarily diffusion-based similar to etch and rinse approach. Group 3 (Xeno V) showed a lower value for hybrid layer thickness (3.86µm) and resin tag length(70.60µm) when compared to strong self-etch adhesive Xeno III and a higher value when compared to mild self-etch adhesive Xeno IV. Intermediate self-etching primers have a pH of around 1.5. Following an "intermediary strong" self-etch approach, the deepest region of the hybrid layer still contains hydroxyapatite, by which the transition of the hybrid layer to the underlying unaffected dentin is more gradual. These adhesives are more acidic than mild self-etch adhesives. Most typical is the two fold buildup of the dentinal hybrid layer with a completely demineralized top layer and partially demineralized base. Following an "etch and rinse" or strong self-etch approach, the transition of the exposed collagen fibril network to the underlying unaffected dentin is quite abrupt. Xeno V contains tertiary butanol as a solvent. The polarity of the water/tertiary-butanol solution would be high enough for activation of acidic monomers, yet adequate for dissolution of less polar ingredients such as initiators. Three distinct acidic monomers would be involved in etching and substrate wetting. The deeper intra tubular infiltration detected with Xeno V could be related to the efficacy of tertiary-butanol as a diffusion promoter. Moreover the methyl groups surrounding the alcohol group in tertiary butanol would prevent addition at the polymerizable groups of monomers, thus increasing chemical stability in comparison with solutions containing ethanol or iso-propanol. In xeno V monomers, acrylic amide groups and inverse methacrylic ester functions were introduced that are claimed to be more stable in an aqueous acidic solution, as compared with acrylic ester functions of methacrylates. Group 4 (Xeno IV) showed the least value for thickness of hybrid layer (2.40µm) and length of resin tag (14.95µm). Xeno IV is mild self-etching system having pH above 2. Mild self-etching systems in general demineralize dentin superficially. This superficial demineralization occurs only partially, keeping residual hydroxyapatite still attached to collagen. Nevertheless, sufficient surface-porosity is created to obtain micromechanical interlocking through hybridization. The thickness of hybrid layer is however, much smaller than that produced by the strong self-etch or etch and rinse approach. One of the reasons for the reduced hybrid layer thickness may be the inability to penetrate through thick smear layers. Also the acidity of the primer could be buffered by the mineral components of the smear layer.

Dentin bonding is a complex problem influenced by many factors. These include variation in the tooth structure, type of the tooth restored, location within a single tooth, the orientation of the fibrils and the tubules at the cavity preparation segments being restored, operator differences, technique variations, differences in ambient conditions etc. Nevertheless, bonding steps such as etching, priming, and bonding during restorative procedures and what happens during these at the microscopic and submicroscopic structural levels are also major importance for optimum results in efficient bonding other parameters such as microtensile bond strength, microleakage, nanoleakage, solvent stability should be considered in assessing the adhesive durability.

Figure 1: Hybrid layer and resin tag length of group-I

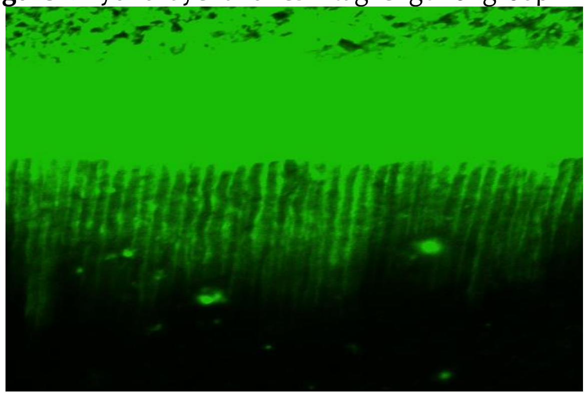


Figure 2: Hybrid layer and resin tag length of group-II

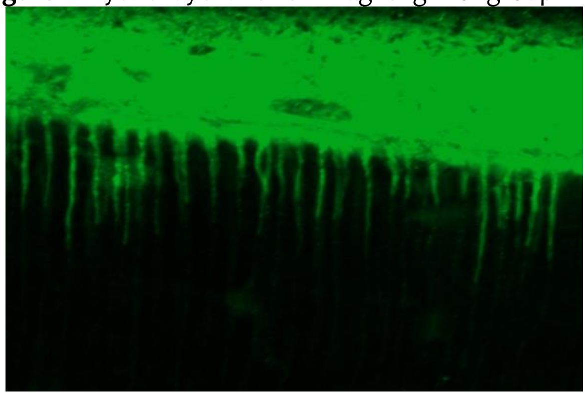


Figure 3: Hybrid layer and resin tag length of group-III

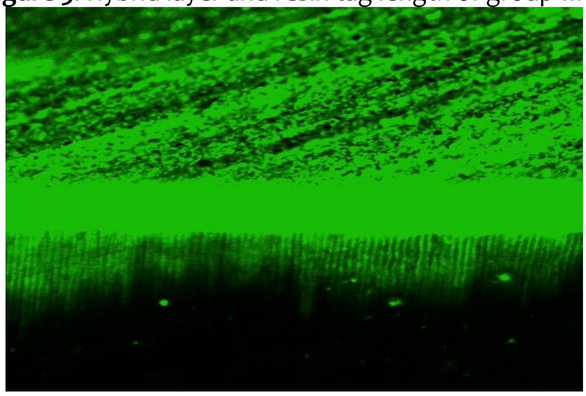
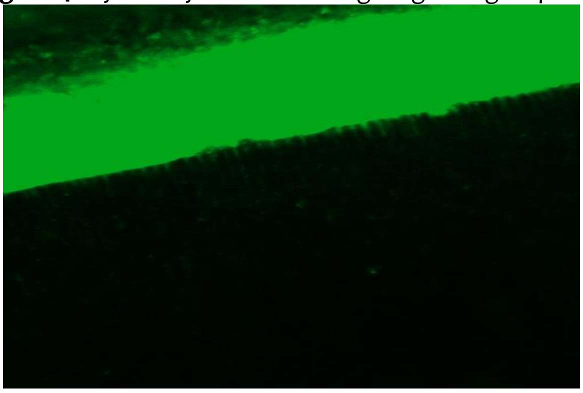


Figure 4: Hybrid layer and resin tag length of group-IV



CONCLUSION

The study conclusion etches and rinse adhesive showed the maximum hybrid layer thickness and resin tag length. Among the self-etching adhesives, strong self-etch adhesive showed the maximum hybrid layer thickness and resin tag length. Mean thickness of hybrid layer formation and resin tag length in etch and rinse adhesive is maximum followed by strong self-etch adhesive, intermediary-strong self-etch adhesive and mild self-etch adhesive. Hybrid layer thickness and resign tag length of mild self-etching adhesive is less when compared to other self-etching primers. Further studies with more parameters must be conducted to evaluate the resin-dentin interface.

Table 1: Multiple comparison of mean thickness (micro meter) of hybrid layer among different groups

Groups	Thickness of hybrid layer (MEAN±SD)
Group-I	5.56±0.74
Group-II	4.56±0.49
Group-III	3.86±0.51*
Group-IV	2.40±0.38*,#

(*P<0.05 significant compared group-I with other groups, #P<0.05 significant compared group-II with other groups)

Table 2: Multiple comparison of mean length (micro meter) of resin tag among different groups

Groups	Thickness of hybrid layer (MEAN±SD)
Group-I	236.16±11.36
Group-II	185.77±5.78*,#
Group-III	70.60±9.06*,#
Group-IV	14.95±4.93*,#,$

(*p<0.05 significant compared group-I with other groups, #P<0.05 significant compared group-II with other groups, $P<0.05 significant compared group-III with other groups)

REFERENCE

1. Ario Santini, Vesna Miletic, Comparison of the hybrid layer formed by Silorane adhesive, one-step self-etch and etch and rinse systems using confocal micro-Raman spectroscopy and SEM, Journal of Dentistry, 2008, 36, 683-691.
2. Abadalla Ali, Franklin Garcia-Godoy, Clinical Performance of a self-etch adhesive in class V restorations made with and without acid etching. Journal of Dentistry, 2007, 35, 558-563.
3. Adam Ostby, Samir Bishara, Effect of self-etchant pH on the shear bond strength of orthodontic brackets, Am J Orthod Dentofacial Ortop, 2008, 134, 203-208.
4. 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 Quintessence, Int, 2005, 36, 511-521.
5. Bitter k, Paris S, Martus P, A Confocal Laser Scanning Microscope investigation of different dental adhesives bonded to root canal dentine, International Endodontic Journal, 2004, 37, 840-848.
6. Fusayama Takao, Nakamura Masato, Non-Pressure Adhesion of a New Adhesive Restorative Resin, J Dent Res, 1979, 58, 4, 1364-1370.
7. Franklin R Tay, David H Pashely, Aggressiveness of contemporary self-etching systems. I; Depth of penetration beyond dentin smear layers, Dental Materials, 2001, 17, 296-308.
8. Goracci Cecilia, Tranchesi Fernanda, Microtensile Bond Strength of Self-etching Adhesives to Enamel and Dentin, J adhes Dent, 2004, 6, 313-318.
9. Gordon J Christensen, Self - etching primers are here, J Am Dent Assoc, 2001, 132, 7, 1041-1043.
10. Genevieve Gregoire, Yasin Ahmed, Evaluation of the enamel etching capacity of six contemporary self-etching adhesives, Journal of Dentistry, 2007, 35, 388-397.
11. Hannig MK, J Reinhardt. Self-Etching Primer vs Phosphoric Acid; An Alternative Concept for Composite to Enamel Bonding, Operative dentistry, 1999, 24, 172-180.
12. Hashimoto M, H Ohno. Fractured surface characterization; wet versus dry bonding, Dental Materials, 2002, 18, 95-102.
13. Ivan Stangel, Thomas Ellis, Edward Sacher, Adhesion to Tooth Structure Mediated by Contemporary Bonding Systems, Den Clin N Am, 2007, 51, 677-694.
14. Jean Camps, David H Pashely, Buffering Action of Human Dentin, J Adhesive Dent, 2000, 2, 39-50.

15. Jorge Perdiago, New Developments in Dental Adhesion, Den clin N AM, 2007, 333-357.

16. Jorge Perdiago, Dentin bonding as a function of dentin structure, Den clin N AM, 46, 2007, 277-301.

17. Kunio Ikemura, Fraklin R Tay, Bonding mechanism and ultrastructure interfacial analysis of a single step adhesive to dentin, Dental Materials, 2003, 19, 707-715.

18. Kerby Re. LA Knobloch, Microtensile Bond Strengths of One-step and Self-etching Adhesive Systems, Operative Dentistry, 2005, 30, 2, 195-200.

19. Karl-Johan M Soderholm, Shear Bond Strength of one 4th and Two 7th Generation Bonding Agents When used by Operators with Different Bonding Experience, J Adhes Dent, 2005, 7, 57-64.

20. Kanchana Waidyasekera, Toru Nikaido, Dinesh Reinforcement of dentin in self-etch adhesive technology: A new concept, Journal of Dentistry, 2009, 37, 604-609.

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