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Original Research

Evaluation of Resin Dentin Interface of a Self-Etch Adhesive in Comparison with a Total Etch Adhesive Using Confocal Microscopy

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Abstract:

Background: *In-vitro* evaluation of the resin-dentin interface using a self-etch and a total etch adhesive system bonded to moist dentin, using confocal laser scanning microscope (CLSM).

Materials and Methods: A total of 40 intact non-carious human premolars stored and sterilized according to Occupational Safety and Health Administration regulations were used for the study. All the teeth, occlusal surfaces, were ground to expose the dentin. 40 teeth were then divided into two groups and treated with different adhesive system. Group 1 (20): Self-etch adhesive system and Group 2 (20); total-etch adhesive system. Rodhamine B dye was mixed with bonding agents and applied on tooth surfaces and cured. CLSM was used to examine resin/dentin interface. The values of resin tag length and thickness of the hybrid layer among the groups were compared and statistically analyzed using Mann–Whitney U-test.

Results: Highest mean length of resin tag was obtained with Group I (110.4 μm) adhesives compared to Group II (35.5 μm) and the thickness of hybrid layer was highest with Group II (4.14 μm) compared with Group I (2.1 μm). A very high statistical significance ($P < 0.001$) was obtained among groups using Mann–Whitney U-test.

Conclusion: 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.

Key Words: Confocal microscope, dentin bonding, hybridization, resin-dentin interface

Introduction

Adhesive technology has evolved rapidly and has brought about a revolution in the field of restorative dentistry.[1] The resin/dentin interface remains the weakest area of tooth colored restorations, despite the significant improvements of adhesive systems and moreover polymerization shrinkage of composites still remains a challenge, a bane and a boon to the dental profession.[2,3]

Adhesive system of Filtek P90 is a two-step self-etch adhesive system containing silane treated silica filler used along with silorane based low shrink restorative composites, that revealed better mechanical properties, but little is known about the formation of hybrid layer and resin-dentin interface.[4,5]

Efficiency of dentin bonding can be evaluated by observing the hybrid layer and resin tag formation.[6] Studies have shown that resin tag and hybrid layer formation would enhance the retention of bond and bond strengths.[7] Confocal laser scanning microscope (CLSM) has been proved to be superior to evaluate resin dentin interface, which generate non-invasive serial optical sectioning of intact specimens and eliminating the artifacts arising with manual sectioning as seen in scanning electron microscopy and transmission electron microscopy techniques.[6,8]

Hence, the purpose of this *in-vitro* study was to evaluate the resin dentin interfaces using a self-etch and a total-etch adhesive system bonded to moist dentin, using CLSM.

Materials and Methods

A total of 40 non-carious human premolar teeth were collected and sterilized according to Occupational Safety and Health Administration and the Centre for Disease Control and Prevention recommendations and guidelines. All the teeth, occlusal surfaces were ground using slow speed diamond disc with copious water supply, so as to expose a flat surface of dentin with an approximate residual dentin thickness of 1.5-2 mm. The teeth were then randomly divided into two groups of 20 specimens each Table 1.

Fluorescent labeling of bonding agent

Rhodamine B dye of 0.1% concentration was used with both the bonding agents before its application to the specimens to enhance the visualization of the distribution of bonding agents.

Table 1: Materials used for the study.

Group	Material	Manufacturer	Composition
Group I Self-etch adhesive system	FiltekP90 adhesive system	3M ESPE	Filtek P 90 system self-etch primer: Phosphorylated methacrylates vitrebond, copolymer, BisGMA, water, ethanol, silane-treated silica filler, initiators stabilizers, HEMA Filtek P90 system adhesive bond: Hydrophobic dimethacrylate, Phosphorylate dimethacrylates, TEGDMA, silane-treated silica filler, initiators, stabilizers
Group II Total etch adhesive system	Adper single bond plus	3M ESPE	Etchant: 35% phosphoric acid Adhesive system: BisGMA, HEMA, dimethacrylates, ethanol, water, photoinitiator and polyitaconic acids and 10% by weight of five nanometer spherical silica particles

TEDGMA: Triethylene glycol dimethacrylate, HEMA: 2-hydroxyethyl methacrylate

Application of bonding agent to test specimens

In Group 1, teeth were treated with self-etch adhesive system (Filtek P90. 3M ESPE). In the first step, Filtek P90 system adhesive self-etch primer was applied with a brush for 15 s with gentle agitation, followed by gentle air dispersion and 10 s of light curing. Then, Filtek P90 fluorescent labeled adhesive bond was applied followed by air dispersion and 10 s of curing. To protect the bonding layer, all the bonded surfaces of Group 1 were restored with 2 mm of resin composite and cured for 40 s using Halogen light curing unit.

Teeth in Group 2 were treated with total etch adhesive system (Adper Single Bond, 3M ESPE). Dentinal surfaces were etched with 35% phosphoric acid (Scotchbond etchant 3M ESPE), for 15 s, rinsed with water for 20 s and left moist by blot drying. Then, using fully saturated brush tip, 2-3 coats of fluorescent labeled Adper single bond plus adhesive was applied with gentle agitation, the surface was gently air dried for 5 s and light-cured for 10 s. All the bonded surfaces of Group-2 were restored with 2 mm of resin composite and cured for 40 s using Halogen light curing unit.

All the specimens were stored with normal saline. After 24 h of storage, the teeth were longitudinally sectioned using a slow speed diamond disc under copious water supply. The dentin/adhesive interfacial region was examined using a CLSM (LSM 510 Meta Confocal Microscope, Zeiss, Germany). The samples were mounted on borosilicate cover glass with a cover slip. Using a ×10 objective, the specimens were illuminated with an Argon laser at 50% intensity using a 514 nm excitation wavelength. Confocal slits were set at 25 μm with a 536 nm long-pass filter. Areas were scanned planoparellel to the cut surface of the specimen. They were analyzed, and thickness of hybrid layer and length of resin tag were measured by means of Image Browser Software (Zeiss, Germany) in μm (Figures 1a and b). The thickness of the hybrid layer and length of resin tag formation were evaluated and scored by one experienced examiner. Results were tabulated and mean obtained.

Results

Statistical analysis was done to compare the values among the groups using Mann–Whitney U-test. Both self-etch and total etch groups had shown the formation and resin tags.

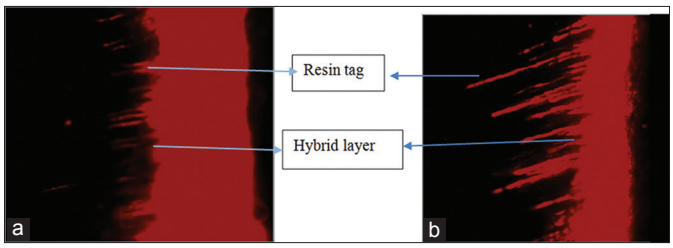


Figure 1: (a) Confocal images of self-etch adhesive, (b) Confocal images of total etch adhesive.

The results showed the mean for resin tag length and hybrid layer thickness in Group I was 35.59 μm and 2.16 μm and in Group II was 110.4 μm and 4.14 μm with a very high statistical significance ($P < 0.001$) using Mann–Whitney U–test (Graphs 1 and 2).

Resin tags obtained with samples were categorized based on homogeneity and base (Table 2). In Group I (self-etch), highest number of specimens belonged to Category 1 and least in Category 3 whereas in Group II (total etch), highest number of specimens belonged to Category 2 compared to Category 1 and Category 3. Good, homogeneous tags and broad base (Category 3) was maximum in total etch group (Table 3).

Total etch in this present study performed well both in the form of resin tag length and hybrid layer thickness than self-etch group.

Discussion

The resin-dentin interface appears to result from the formation of an interdiffusion zone (hybrid layer) in which resin infiltrates the interfibrillar spaces of a collagen meshwork exposed by the acidic pretreatment. Resin tag development into the opened dentin tubules additionally contributes to the eventual bond.[9] In the present study confocal microscopy was chosen for analyzing these adhesive junction zones because it is able to optically penetrate a structure without destruction at a maximum depth of about 100 μm and offers superior images of the resin dentin interface.[10]

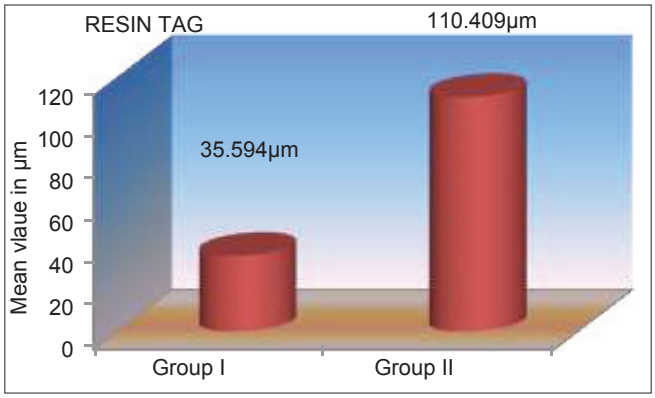
The mean value of resin tag length in self-etch group was 35.5 μm and total etch group was 110.4 μm. Baweja *et al.*,[11] obtained similar result using confocal microscopy on contrast to the mean length of resin tag for total etch (prime and bond

Table 2: Criteria applied for examination of the resin tag based on length, homogeniety and base.

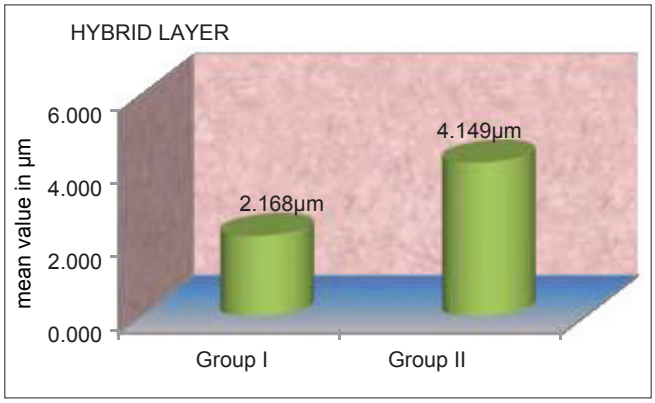
Tags	
Category 1	Low; inhomogeneous tags, narrow base
Category 2	Medium; quite homogeneous tags, average base
Category 3	Good; homogeneous tags, broad base

Table 3: Number of specimens included under each criteria in Group I (self-etch) and Group II (total etch).

Group	Category 1	Category 2	Category 3
I	12	7	1
II	6	9	5



Graph 1: The mean and standard deviation of the resin tag length in Group I (self etch) and Group II (total etch).



Graph 2: The mean and standard deviation of hybrid layer thickness in Group I (self-etch) and Group II (total etch).

NT) was 239.2 µm which is higher than the mean value of the total etch resin tag used in the study. The present study used ethanol based solvent in contrast to prime and bond NT which has acetone-based solvent. Another study by Oliveira *et al.*[12] and Machado *et al.* (2009) reported a lower mean value of resin tags for self-etch and total etch group.[13]

The hybrid layer thickness observed in our study for total etch group ranged from 1.7 to 6.03 µm (mean value 4.14 µm) and self-etch was in the range of 1.3-4.14 µm (mean value 2.1 µm). Study by Mohan and Kandaswamy[14] on resin dentin interface showed similar mean hybrid layer thickness for single bond adhesive using confocal laser microscopy. Our study corroborates with the study of Oliveira *et al.* (2009) and Sundfeld *et al.* (2005) for evaluation of hybrid layer thickness in self-etch adhesives.[12,15]

The possible explanations for the better performance by total etch in terms of resin tag length (1) Demineralising action of the strong etchant (phosphoric acid) used in total etch group and subsequent rinsing of the reaction products,[16] (2) The moist bonding technique prevents the collagen fibers from collapsing (Mohan and Kandaswamy).[14] Hence moist bonding is preferred, (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. Compare these points to the present results, total etch performed better than self-etch in terms of resin tag length.

Maximum mean value of the hybrid layer for total etch is attributed to the complete removal of the smear layer and smear plug and consequent dentin demineralization promoted by the 35% phosphoric acid. Since the conventional adhesive system exhibits a more acidic pH (0.6) and a higher pKa value than the self-etching adhesive system (Ph = 2.7). Strong acids also increase the depth of dentin demineralization with greater exposure of collagen fibers and are subsequently infiltrated by monomers with polymerization. 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 the self-etching system.[15]

Thicker and homogenous hybrid layer and good homogenous resin tag were formed by the total etch group in our study that is in accordance with the study by Baweja *et al.*[11]

LS system adhesive bond contains silane-treated silica filler. Addition of fillers increases the viscosity of the adhesive, moreover higher concentration of fillers are normally seen in the openings of the dentinal tubules conceivably reducing the penetration of adhesive monomer in the dentinal tubules.

Though total etch performed superior to self-etch in evaluation of resin dentin interface review by Van Meerbek *et al.* (2001) on adhesion revealed, rather than the actual length of the resin tag, its seal at the tubule orifice by the base of the tag contributes the retention and sealing effectiveness because it is protective when bond fails at the bottom or top of the hybrid layer.[3]

The confocal studies only give an insight into resin-dentin interface but don't give the degree of conversion of monomers and chemical nature of the interface that plays a major role

in adhesion. Hence, further clinical research and laboratory investigations on the bond strength are required to investigate their effectiveness.

Conclusion

We conclude by evaluating the resin-dentin interface pertaining to length of resin tags and hybrid layer thickness that the total etch group (adper single bond) to be superior compared to self-etch group (Filtek P90).

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