ORIGINAL ARTICLE

A Comparative Evaluation of Tensile Bond Strength and Hybrid Layer of Three Generation Bonding Agents by Scanning Electron Microscope (An *In-vitro* Study)

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ABSTRACT

This study was done to compare the tensile bond strength of three different generation bonding agents to dentin using one composite resin and scanning electron microscopic study (SEM) of hybrid layer.

In this study 36 non carious human molar teeth were selected. 30 teeth were used for evaluating tensile bond strength and were divided in to three groups. Six teeth were used for hybrid layer evaluation also divided into three groups. Group 1 specimens 10 teeth were etched with 37% phosphoric acid and Scotch bond multipurpose adhesive was applied. Group 2 specimens 10 teeth were etched with 37% phosphoric acid and 3M Single bond was applied. Group 3 specimens 10 teeth were treated with Prompt-L-Pop. Composite material (3M-Hybrid-Z100) was applied incrementally and cured for 40 seconds each. Specimens were then stored in distilled water before testing. Two teeth from each group were cross-sectioned to obtain 1mm thick dentin disks for SEM evaluation. Adhesives were applied as mentioned above. Composite was applied over these discs and light cured for 40 seconds. Samples were stored in distilled water at 37^{0}c for two weeks; these disks were fractured with chisel for cross-sectional viewing

Dentin conditioning with single bond (5th Generation) revealed better bond strengths as compared to scotch bond multipurpose (4th generation) and Prompt-L-Pop (6th generation). SEM evaluation of hybrid layer reveals that Single bond has shown a thicker hybrid layer comparing to other adhesives.

Key words: Adhesives, Bond strength, Hybrid layer.

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The introduction of tooth-colored composite represented a leap forward in the development of adhesive system. Adhesion to dental tissue is a fundamental requirement prior to the insertion of tooth colored restorative material(1).

Bonding of resin to dentin is far more difficult and less predictable, due to the specific properties of human dentin, such as the tubular structure and its intrinsic wetness. The bonding mechanism of recent bonding agents is based on penetration of ambiphilic molecules into acid etched dentin. Water-chasing solvents, such as acetone or ethanol, are commonly utilized to facilitate penetration of monomer and to obtain a direct contact of resin with collagen fibers, which result in a mixed zone of polymerized resin and entangled collagen fibrils, called the hybrid layer(2).

To better describe the adhesive or bonding agents the term "generations" was established. 4th generation adhesives

involves a three step process, conditioning (or etching), priming and finally application of bonding agent. 5th generation involves two-step process but known as "one bottle adhesives" and have only single component for both the priming and bonding stages. A different philosophy is advocated by "self etching primers". These prime enamel and dentin simultaneously without rinsing.

Self-etching primers hybridize dentin up to 2µm and have been reported to withstand stresses from polymerization shrinkage clinically. These materials have the ability to dissolve hydoxyapatite partially, both within the smear layer and dentin surface, resulting in a resin infiltrated zone with entrapped minerals. The simultaneous treatment of enamel and dentin with one material in one application is a major advancement towards simplification of the clinical procedure and reduces the possibility of error during the application of current adhesive materials.

The ultimate simplification is a new bonding concept (6th generation) known as "all-in-one" involving only one step. A new all-in-one adhesive has been reported to result in enamel bond strengths comparable to those obtained by total-etch multi- step adhesives(3).

Material and methods

A total of 36 freshly extracted caries free, unrestored human molars were selected and stored in distilled water. The occlusal surfaces of these teeth were ground using a water cooled diamond disc mounted on an air motor hand piece until all occlusal enamel was removed. This resulted in exposure of flat dentin surface, with enamel at periphery. Horizontal indentations were placed with fissure bur on the radicular portion of the specimen to attach the specimen to the resin block. The teeth were embedded into self-curing resin leaving the dentin surface exposed. The specimens were randomly divided into 3 groups of 10 teeth each. Group 1 the occlusal surfaces of specimens were treated with 37% orthophosphoric acid for 15 seconds. The etchant was rinsed off with water and surface blotted, leaving the tooth moist. Scotch bond primer was applied to the bonding surface, dried for 4 seconds with gentle air pressure as recommended by manufacturers. Scotch bond multipurpose adhesive was applied to the primed surface and light cured for 10 seconds. A hollow polyvinyl cylinder with inner diameter of 5 mm and height of 6mm was placed on the treated dentine surface almost at the center of the specimen and composite resin was condensed to 2mm thickness and light cured for 40 sec. Another 2mm of uncured resin was then placed.

A 26-gauge ligature wire was twisted at one end and a loop formed at other end and the twisted end was placed inside the 2mm of uncured resin .The composite resin was then light cured. This was followed by placing another 2mm of composite resin to stabilize the wire. Following complete curing, polyvinyl cylinders moulds were cut and removed. In Group 2 the occlusal surface of specimens were treated with 37% orthophosphoric acid for 15seconds. Etchant was rinsed off with water and surface blotted, leaving the tooth surface moist. Two consecutive coats of adhesive (3M Single Bond) were applied and air dried for 2-5seconds, as recommended by manufacturers. The adhesive was light cured for 10seconds.The composite resin was inserted and cured in the same manner as in Group I.

In Group 3 the occlusal surfaces of the specimens in this group were treated with self-adhesive composite material (Prompt-L-Pop) as per manufacturer's recommendations. Prompt-L-Pop was applied to entire surface of the tooth and rubbed for 15seconds. Then carefully an air stream was applied to create an even, slightly shiny film. Light cured for 10 seconds as recommended by manufacturers. Tensile strength was evaluated by Instron machine model 4301. A crosshead speed of 0.5mm/minute was selected and tensile load was applied on the specimens until the composite cylinders were dislodged from the surface of tooth. The values obtained were in 'Kg' which was converted in MPa.

For SEM study, 6 recently extracted human molars stored in distilled water at 4˚C were used. These teeth were mounted in blocks of acrylic resin and the occlusal third was removed using slow speed cutting air motor equipped with a diamond-impregnated disk under water coolant with copious water. Subsequently remaining surface was polished with 180 grit, 240 grit and 600-grit silicon carbide sand paper until no enamel remained. Teeth were then cross-sectioned to obtain 1mm thick dentin disks. Outer surface of each disk was polished with wet 600 grit silicon carbide sand papers to create a uniform smear layer. With the help of diamond disk, 1mm of horizontal section of dentine were taken. Six specimens were taken and divided in to three groups. Specimens were treated with etchant and adhesives as mentioned above in the three groups. 2mm of composite was applied over these dentine discs and light cured for 40 seconds. After preparation, these specimens were stored in distilled water at 37°C for two weeks. These disks were fractured with chisel for cross sectional viewing. All specimens were than immersed in 5% hydrochloric acid for 30 seconds and than washed for 30 seconds under running water. Sections were then transferred to 70% ethanol and dehydrated in increasing concentration of ethanol for 10 second each. These sections were mounted on aluminum stubs with silver paint and sputter coated with gold-palladium, then examined with a Philips scanning electron microscope using an acceleration voltage of 15Kv at magnification of 1000x.

Statistical analysis were done using kruskal-wallis one- way ANOVA for variable Tensile Bond Strength which were obtained comparing significant differences among
P-value <0.001. Tensile bond strength values in MPa

Table 1:

	GROUP 1	GROUP 2	GROUP 3
SPECIMEN NO. 1	18.67	19.52	10.20
SPECIMEN NO. 2	18.05	18.41	10.64
SPECIMEN NO. 3	19.89	16.21	9.8
SPECIMEN NO. 4	17.65	17.85	11.5
SPECIMEN NO. 5	17.70	21.48	12.54
SPECIMEN NO. 6	19.17	18.14	10.40
SPECIMEN NO. 7	18.14	19.63	11.29
SPECIMEN NO. 8	16.04	20.87	12.1
SPECIMEN NO. 9	20.18	22.23	11.75
SPECIMEN NO. 10	18.15	16.91	12.48

Results

The tensile strength data are reported in Table 1, Table2, Table3 and SEM analysis are presented in Table 4 and Figs. 1,2,3, 4,5,6.

The mean values of group 2 were higher than the mean values of group 1 and group 3. The mean values of group 2 were higher than group1 but were not statistically significant.

SEM evaluation of hybrid layer showed that Prompt-L-Pop has produced a thinner hybrid layer compared to other adhesives and it was irregular and non uniform in thickness with some areas being extremely thin while others were relatively thick.

Table 3: Statistical Analysis of Difference in Tensile Bond strength in between groups

Groups	1	2	3
1	-	NS	SS
2	NS	-	SS
3	SS	SS	-

SS = Statistically significant
NS = Non significant

Table 4:

Groups	Width of Hybrid layer in µm Min.	Max.
1	1	5.5
2	0.5	7
3	0.2	2

Discussion

Freshly extracted caries free human molars were used, which were stored in distilled water. Molars were preferred in this study as flat dentin surface could be prepared which would give a wider area of dentin to be treated and bonded to resin substrate. With reference to previous studies the specimens of group l and 2 were etched with 37% phosphoric acid. Kimochi *et al* in 1999 suggested that 37% phosphoric acid to be used in order to attain high tensile bond strength(4). Barkmeier and colleagues in 1986 had observed that there was no difference between 15seconds acid application and 60 seconds

Table 2. One Way

Descriptives

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	N	Mean	Std. Deviation	Std. Error	95% Confidence interval for mean Lower Bound	Upper Bound	Minimum	Maximum
Group 1	10	18.3640	1.1980	.3788	17.5070	19.2210	16.04	20.18
Group 2	10	19.1250	1.9764	.6250	17.7112	205388	16.21	22.23
Group 3	10	11.2700	.9724	.3075	10.5744	11.9656	9.80	12.54
Total	**30**	**16.2530**	**3.8593**	**.7046**	**14.8119**	**17.6941**	**9.80**	**22.23**

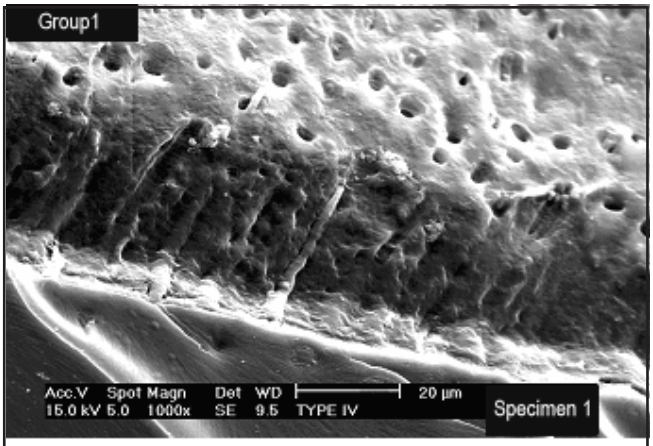

Fig 1: SEM Photograph of hybrid layer in group 1 (specimen 1)

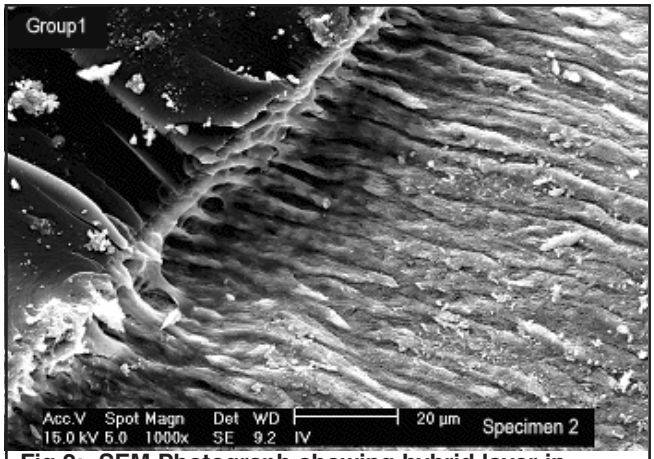

Fig 2: SEM Photograph showing hybrid layer in group 1 (specimen 2)

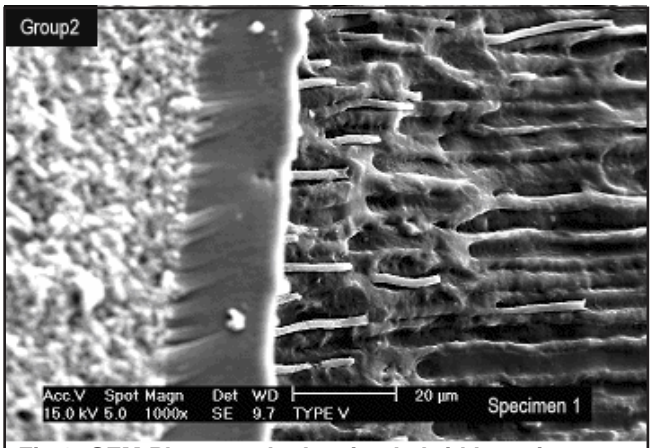

Fig 3: SEM Photograph showing hybrid layer in group 2 (specimen 1)

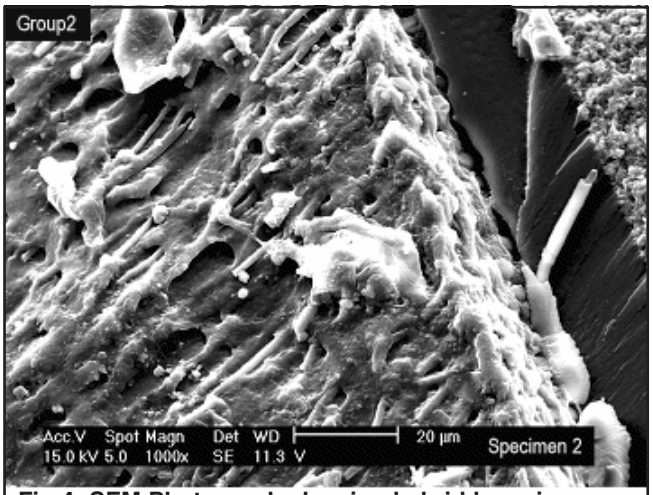

Fig 4: SEM Photograph showing hybrid layer in group 2 (specimen 2)

application, even though there was variance in depth of penetration but SEM evaluation had revealed that there was no difference in pattern of demineralization between 15 and 60 seconds acid conditioning time(5). "3M ESPE Z100" composite material was used because it is the most commonly used material for anterior and posterior restorations. 5mm diameter of polyvinyl mould were used so that it covers the maximum surface of tooth, 6mm height was kept so that it gives sufficient amount of composite material to hold the ligature wire. Poly vinyl cylinders were used to avoid uncured resin as light can pass through it and are easy to remove. Composite was placed incrementally. Eick and Welch in 1986 suggested that applying the composite resin incrementally could reduce polymerization shrinkage stresses(6).

Curing period of 40 seconds was observed to avoid uncured resin, as this would cause bonding failure at an undesired site during testing. Specimens were kept in distilled water before testing to simulate the oral conditions. A tensile type of test was applied to test the bond strength. Four factors normally influence the final strength of the union of composite resin to the tooth surface, namely Wet ability, stress setup by the setting contraction of the resin, filler composition of composite resin, tensile strength of the material. Out of these factors, the role of tensile strength of the material when bonded with different generation bonding agents was considered in this study(7). Results of the present study shows that out of three groups, group 2 Single bond (5th generation bonding agent two step adhesive) produced highest tensile bond strength (table1) compared to the other groups. The self-etching one step 6th generation adhesive yielded the least values of bond strength. These results correlate with the results of previous study done by Ana Maria Spohr and Lourence Correr Sobrinho, etal (8). One bottle adhesive system 5th generation bonding agents obtains higher bond strength values than the self-etching adhesive 6th generation bonding agent upon shear and tensile tests(9). Study done by Asmussen and Munskgard (1985) on bonding of restorative resin to dentin has inferred that using adhesive based on HEMA and propionic aldehyde and HEMA and glutraldehyde, a higher tensile bond strength is achievable(10).

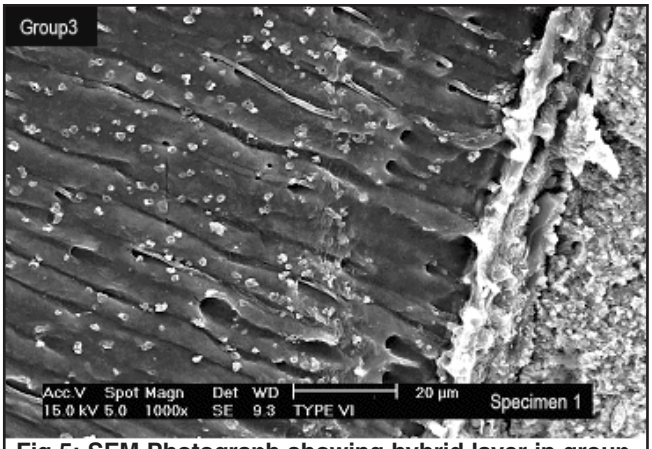

Fig 5: SEM Photograph showing hybrid layer in group 3 (specimen 1)

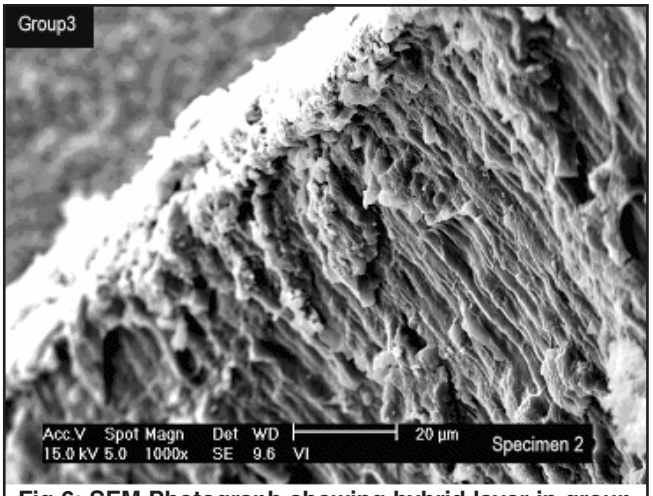

Fig 6: SEM Photograph showing hybrid layer in group 3 (specimen 2)

A study done by Perdigao J. revealed that high bond strengths obtained with 3 M Single bond. The relatively high bond strengths consistently obtained with (group1 and group 2) may be a consequence of being a polyalkenoic acid-based adhesive. Polyalkenoic acid-based adhesives have been associated with resistance to degradation in humid environment. Additionally there may be intrinsic stress-relaxation ability in Ca-polyalkenoic acid-based adhesives(11).

The single bond is better than multi step in forming more consistent bonding with fewer porosities and gaps. The short time between conditioning and bonding might prevent collapse of collagen of fibril network and allows better penetration(12). Comparing group I and group 2 with group 3, Scotch bond Multipurpose and Single bond showed higher bond strengths than Prompt-L-pop which might be because of smear layer being removed in scotch bond multipurpose and Single bond with 37% phosphoric acid whereas in case of Prompt-L-Pop the smear layer gets dissolved in it and is not removed. Smear layer plays an important role in bonding. Normal thickness of smear layer is 0.5-5.0 μm; it acts as a barrier that decreases permeability of dentin. This can be considered as an impediment that must be removed so that resin can bond to underlying dentinal substrate(13). Studies have shown that phosphoric acid etching prior to the application of Prompt-L-Pop as conventional primer, followed by bonding resin resulted in an increase in bond strength(14).

This may be the cause of lower bond strength in this study, as group 3 specimens were not etched. For SEM evaluation, samples were prepared as suggested by

Andia-Merlin; Nakabyashi *et al*(15,16). After specimen preparation, dentin disks were left in distilled water at 37°C for two weeks then fractured perpendicularly to the bonded surface to obtain two hemi disks.

The fractured specimens were treated with 5% Hydrochloric acid (HCl) for 30 seconds and than washed in running water for 30 seconds. Treating samples with HCl for 30 seconds makes identification of dimensions of hybridized dentin clearly by removing mineralized dentin below the hybrid layer there by increasing the surface relief (16). Increasing concentrations of **Ethanol 70%-100%** was used for dehydrating the specimens, and making it suitable for SEM analysis. For evaluation of **"hybrid layer",** Scanning Electron Microscopic study was done. Due to short wavelength of electron beams, SEM offered sufficient resolution to identify the formation of few micrometer wide hybrid layers. SEM images of hybrid layer have steadily increased in number and improved in quality, making SEM the most popular and easiest tool to morphologically examine bonding mechanism(17). SEM evaluation of hybrid layer reveals that Single bond has shown a thicker hybrid layer comparing to Prompt-L-Pop and Scotch bond multipurpose Figs. 1,2,3,4,5,6.

It was observed that thicker hybrid layer did not provide higher bond strength(12,18) Miyasaka and Nakabyashi (1999) reported that as the hybrid layer thickness increases from 1-3μm, the tensile strength decreased from 22–10 ± 3MPa(19). M. A.Vargas *et al* in their study on TEM evaluation of self-etching adhesives resin dentin interfaces inferred that all self etching adhesives showed evidence of hybrid layers that vary in thickness. Prompt-L-Pop showed hybrid layer of 2-3μm thick(20). Genevieve L. Gregorie inferred that the hybrid layer formed by all self-etching products is thin. This thinness is due to a decrease in acid concentration as it infiltrates dentin and meets moisture(21). Nakabayashi (1995) quoted that there is a danger of misinterpreting high bond strengths obtained with conventional bond-testing techniques as being indicator of high quality of hybrid layer(16). Prati C *et al* (2000) in his study inferred that there was no correlation between the thickness of hybrid layer and bond strength(22).

J Perdigao and others stated that the thickness of hybrid layer and its influence on bonding is still uncertain. They suggested that dimensions of hybrid layer may be taken as an indicator of the strain absorbing capacity of the corresponding interface. The elastic buffer could be of utmost importance for absorbing the stress originated from composite resin polymerization shrinkage. Nevertheless no correlation was established between thickness of hybrid layer and bond strength(11).

Sakoolnamakara *et al* in (2002) inferred that there was no correlation between the hybrid layer thickness and bond strength. The quality of hybrid layer may be more important with respect to bond durability(14).

Some studies have revealed that there is a correlation between Hybrid Layer thickness and bond strength, and others reveal that thickness of hybrid layer doesn't play any role in bond strength. Considering this controversial issue of hybrid layer thickness-Bond strength relationship, further research is necessary to arrive at any concrete conclusion.

Conclusion

Current development in adhesive systems has focused on simplifying the application methods by decreasing the steps required for placement. Recent advances in chemistry of dentin bonding systems have improved bond strengths. Prompt-L-Pop was initially introduced for use with compomers. The manufacturers have recently expanded the indications of Prompt-L-Pop, suggesting its use even for bonding composite. The results of this study has acknowledged that dentin conditioning with 37%.

Phosphoric acid followed by application of conventional

adhesive (Single bond, 5th generation), provided consistently better bond strengths as compared to self etching adhesive (6th generation) system. Combination of etchant, primer and adhesive resin into all-in-one adhesive is advantageous, in that it reduces the application time as well as errors that may occur during each bonding step, but as far as bond strength values are concerned, there is still scope for enhancement.

The relation ship between thickness of hybrid layer and bond strength remains a controversial issue and requires further research to reach any distinct conclusion.

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