Microtensile bond strength of Resin composite to Normal dentin and Caries - affected dentin with total-etch and self-etch bonding adhesives before and after thermocycling

Dr. Deepti Rao M and Dr. R Nageswar Rao

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Abstract

Objectives: To measure the microtensile bond strength of total-etch and self-etch adhesives to intact and caries affected dentin. To check the effect of thermal cycling on the bond strength of these adhesives to caries affected and intact dentin.

Materials and Methods: Thirty extracted carious human molars were assigned to two groups according to adhesive used i.e Adper Single Bond (Total etch) with P90 (Self-etch). The occlusal surface of teeth will be sectioned to expose dentin and built up with Filtek 90 composite (3M) to a 3 mm height. The teeth were then sectioned to obtain rods of 6mm length and 1mm width and 1 mm depth with dentin-composite interface located at the centre. The specimens were examined under microscope to separate caries–affected dentin and unaffected dentin into two groups. Half the specimens from each group were subjected to 3000 thermo cycles (5 deg Celsius to 55 deg Celsius). All specimens were subjected to microtensile bond strength testing in a special apparatus attached to the Universal testing machine. Results were recorded and data was statistically analyzed with Students unpaired t-test.

Results: Students t test showed that the values had a statistically significant difference observed between total etch (T.E) i.e Adper Single Bond 2 and self etch (S.E) i.e P 90; caries-affected dentin (CAD) and normal dentin (ND); with thermocycling and without thermocycling.

Interpretation and Conclusion: The present study concludes that total-etch has a higher bond strength compared to self-etch. Bond strengths are higher to normal dentin than caries-affected dentin. Thermocycling lowers the bond strength values of all the groups.

Keywords: Caries-affected dentin, normal dentin, Microtensile bond strength, total-etch dentin bonding agent, self-etch dentin bonding agent, thermocycling

1. Introduction

The increasing demand for esthetic restorations has generated intensive research of adhesive materials. Successful adhesion to dental hard tissues is a fundamental requirement prior to the insertion of resin - based composites [1]. Dentin adhesives are widely used in clinical dentistry and improved or new versions are being constantly introduced which are claiming to offer advantages over their predecessors [2]. Since Buonocore, a number of new adhesive systems have been developed in an attempt to reduce the steps & simplify clinical bonding procedures [3]. One approach employs the total-etching technique, which simultaneously removes the smear layer from both enamel & dentin surfaces, followed by application of a 1- bottle primer & adhesive. As demineralised collagen fibril mesh is used as the bonding substrate, a wet bonding technique is required to ensure its full expansion. The need for a moist dentine surface in complex cavity preparations often create overwet and underwet regions in the same tooth, making bonding to dentin with these adhesives very technique sensitive.

The second approach is use of self-etching primers. Bonding mechanism is based on simultaneous etching & priming of the smear covered dentin using an acidic primer followed by application of an adhesive resin. These eliminate separate acid etching & rinsing steps & simplify bonding as the problem of over drying or over- wetting the dentin is eliminated.
This reduces the potential for post-operative sensitivity & bonding problems associated with movement of dentinal fluid through patent dentinal tubules. The technique sensitivity associated with bonding to a dehydrated collagen matrix is also eliminated. These systems also provide a faster application due to reduced number of components and application steps [4].

Bonding to enamel is primarily based upon mechanical interlocking of the bonding resin with the created micropores on the enamel surface. Bonding to dentin is more complex due to inherent characteristics & morphological features of dentin with different chemical & histological composition. Dentin has tubules running through it, carrying dentinal fluid from the pulp. This causes bonding process to be more challenging & unlike enamel, a primer is always necessary when bonding to dentin to form the hybrid layer [3].

The development of recent adhesive restorative materials has brought about changes in the philosophies of cavity preparation. These have been replaced by more conservative techniques with removal of only infected, permanently damaged carious tissue. But the bacteria-free, caries-affected dentin / sclerotic dentin are preserved and disease progression is prevented. Most adhesion studies are performed on normal dentin, although clinically, most substrates are not normal dentin. Clinicians must deal with variants like caries-affected dentin, cervical sclerotic dentin or even intrinsic differences between coronal & radicular dentin.

Several other factors are known to effect the tensile bond strength of dentin bonding agents such as long term storage, thermal stress, tooth flexure, simulated pulpal pressure, dentin substrate variations & 3- dimensional cavity preparation to name a few [6]. Also the occlusal forces applied to a restoration may be complex & made up of a combination of forces such as shear, tension, compression & flexure with tensile stresses being the most common. Marginal percolation is initiated, in part, by the difference in the coefficient of thermal expansion of the tooth and the restoration and by the thermal expansion of fluids occupying the tooth/restoration interface. (Nelsen et al.) Hence to study these effects thermocycling is done in the present study. The efficiency of these simplified bonding systems is controversial and practically all published reports have used normal dentin as bonding substrate. However, most clinical adhesive procedures involve altered forms of dentin [3].

Conventional testing methodologies are not applicable to such clinically relevant substrates due to the limited size & irregular shape of abnormal dentin [5]. Accordingly, it follows that no single test can satisfactorily predict the intraoral behaviour of an adhesive system. Adhesion analysis of dentin bonding agents has been performed by numerous mechanical testing methods including shear bond strength, tensile bond strength, microleakage & contraction gap size measurements. Agreement on test standardization is complicated by the enormous number of test variables involved & the variable nature of the heterogeneous dentin [6]. The development of microtensile test method, that utilizes specimen cross-sectional areas of approximately 1mm², has allowed the determination of bond strengths of several bonding systems to caries-affected dentin [3].

Thus this study aims to determine the microtensile bond strength of caries-affected and normal dentin using total-etch & self-etch adhesives before and after thermocycling. This may provide an added insight into understanding the essentials of the fundamental mechanism of adhesion of polymeric biomaterials to dentin.

1. Objectives
   1. To measure the microtensile bond strength of total-etch and self-etch adhesives to intact and caries affected dentin.
   2. To compare the bond strengths to caries-affected and normal dentin
   3. To check the effect of thermal cycling on the bond strength of these adhesives to caries affected and intact dentin.

2. Materials and Methodology
   2.1 Sample Selection: This study utilised freshly extracted human molars (n=30) previously stored in 3% chloramines solutions. All teeth had Class 1 occlusal caries, and were not pulpally involved. (Fig 1 A, B) The crown portions were horizontally sectioned at a level 1mm below the dentino-enamel junction using the slow speed diamond disc with intermediate water cooling to expose the flat dentinal surface. Both carious & normal dentin was present on the same surface. The exposed dentin surfaces were examined visually and their surface texture was tested with a spoon excavator. Surfaces with soft, discoloured dentin were considered caries-infected and were exclude ed. Hard, brown dentin was considered as caries-affected, while light-yellow dentin was considered to be sound. Dentin surfaces were further examined using a light microscope to eliminate specimens having either enamel or pulp tissues.

![Fig 1: (A) Thirty molars with occlusal caries not involving the pulp were used. (B) The crown was sectioned at the level of the DEJ to expose parts of Normal Dentin (ND) and Caries Affected Dentin (CAD)](http://www.oraljournal.com)
2.2 Grouping of Samples: The selected teeth were randomly divided into 2 groups of 15 teeth each (Fig 2), according to bonding agent used i.e.

Group I- Total-etch group (n=15 teeth);
Group II- Self-etch group (n=15 teeth)

2.3 Bonding of Samples
The flat dentinal surfaces of each group of teeth were then built up with composite resin, after application of the respective dentin bonding agents. The adhesives were applied according to manufacturer’s instructions. For Group I, 15 teeth were bonded using a 2-step total-etch bonding agent, Adper Single Bond 2 (3M ESPE, St Paul, MN, USA). Firstly, an etchant was applied to enamel & dentin. After an interval of 15 seconds, the surface was rinsed with water for 10 seconds. Excess water was blotted using a cotton pellet. The criteria for dryness were considered that the surface should appear glistening without pooling of water.

Immediately after blotting, 2-3 consecutive coats of adhesive was applied for 15 seconds, gently with a saturated applicator brush. It was gently air thinned for 5 seconds and light cured for 10 seconds using a quartz halogen light curing device. A universal restorative composite, Filtek Z350 (3M ESPE) A shade was used for core build-up. It was built incrementally to form a bulk of composite of 3mm height using Teflon coated cement spatulas and light-cured for 20 seconds each.

For Group II, 15 teeth were bonded using a 2-step self-etching dentin bonding agent, P90 System Adhesive (3M ESPE, St Paul, MN, USA). This system consists of a self-etching primer and bond in dosing bottles. Primer was applied to the entire surface with an applicator tip, in a massaging action for 15 seconds over the entire area and air dried gently. It was then light cured for 10 seconds.

Similarly, the Bond (adhesive) was applied. After bonding, immediately the surface was built-up using Filtek P90 (3M ESPE), a posterior, radiopaque, shrink free, silorane-based composite. A composite core of 3 mm was built incrementally and light cured for 20 sec each. Filtek P90 & P90 System Adhesive was used only in combination with one another as per the manufacturer’s instructions.

2.4 Sectioning
These teeth were then stored at 37 degrees C for 24hours in distilled water. They were then sectioned with a diamond disc...
to obtain specimens, each of 1mm thickness and 6 mm length. (Fig 2 A,B) The sections were left attached to the remainder of the tooth for further sectioning to obtain rods 6mm long and 1x1 mm in thickness with dentin-composite interface located at the centre. The specimens were smoothed using the Arkansas stone. This also helped in achieving the required dimensions. The dimensions were measured with the help of a screw gauge. In each group (Group I =15 teeth; Group II =15 teeth), each tooth was sectioned to obtain 4 specimens per tooth on an average. Hence, each group consisted of 60 rods for each bonding agent i.e. (Group I = 60 rods; Group II = 60 rods) (Fig 2 C, D). The specimens were then examined under a light microscope using 40 x magnifications to separate caries–affected dentin and normal dentin. It was divided into two equal subgroups (n=30) depending upon whether it was normal dentin (ND) or caries–affected dentin (CAD). Half the specimens from each subgroup group (n=15) were subjected to thermocycling.

2.5 Thermocycling
Thermocycling was performed in the Mechanical Engineering Department at the S.D.M Engineering College, Dharwad. The apparatus for thermocycling consisted of 2 water baths (Fig 3 A, B, C, D) which were maintained at two specific temperatures ranging from hot to cold. One water bath was maintained at 5 degree Celsius, by constant addition of ice cubes and the other water bath was maintained at 55 degree Celsius, by using a heating apparatus connected to a thermostat. Both had a thermometer inserted into the respective baths and the change in temperature was manually controlled to maintain the required temperatures. Half of the sections from each of the 4 subgroups i.e. (n=15) were separately tied in gauze pieces and placed in each bath, with a dwell time of 30 seconds and a 10 second transfer period between the two baths. A stopwatch was used so as not to exceed the time limit. This was done for 3000 cycles manually.

2.6 Microtensile Testing: All the specimens (n=120) then were loaded onto a jig (Custom-made at Nettur Technology & Tools Fabricators (NTTF)) (Fig 4A) and underwent the microtensile testing using the Universal testing machine (Instron) (BISCO Inc, Schaumburg, IL, USA) (Fig 4B). Each specimen was glued to the jig using adhesive, Fevi Quick and left for 2 minutes to dry. These were then mounted onto the Universal testing machine. Tensile load was applied until specimen failure at a speed of 1mm per minute. Maximum load applied and failure load was recorded for each specimen and the microtensile bond strength was calculated. Means and standard deviations were determined for each group and data were statistically analyzed using students t – test.

Fig 3: Thermocycling apparatus assembled and being carried out in (A) Beaker of water on a heating apparatus connected to a thermostat. B: thermometer shows a temperature of 55 Deg C achieved and maintained. (C) Beaker filled with ice and a thermometer inserted to check the temperature acheived: Thermometer shows temperature of 5 Deg C achieved.

Fig 4: (A) An apparatus jig to position the Dentin-Composite resin section was custom designed and milled. It had a slot to fit the sample size of 1mm x1mm x 6mm. Length of the jig was made of an arbitrary 5inches to fit into the Instron. (B) Universal Testing Machine, Instron which allows the jig to be placed and gives a digital recording of the value of at which the bond fracture takes place.
3. Results

On bond strength testing, the maximum load at which the failure at the resin-dentin interface occurred was recorded digitally. The values obtained were in kiloforce / mm² and were then converted into Mega Pascals. The values were arranged in tabular columns and subject to Blom’s proportional estimation formula to check the normal distribution parameters. Since, all the values were found within the normal distribution, a Students’ t’ test was done for statistical analysis.

In our study, (Refer Table 2) the values obtained by Adper Single Bond 2 (Total Etch) for ND with thermocycling were 25 MPa, whereas without thermocycling it was 28 MPa. For CAD, it showed bond strengths of 17 MPa with thermocycling and 20 MPa without thermocycling (p=0.00). With P90 (Self-etch), ND showed bond strengths of 15 MPa with thermocycling and 18 MPa without thermocycling. For CAD, in the same group, with thermocycling a value of 12 MPa was obtained whereas without thermocycling, the bond strength achieved was 15 MPa mean value (p=0.00).

Table 2: Results of Maximum Loading on the Sample expressed in MPa

<table>
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<th>Class</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
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<tr>
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<td>28.8533</td>
<td>3.1412</td>
<td>.8110</td>
</tr>
<tr>
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<td>15</td>
<td>25.4200</td>
<td>1.9266</td>
<td>.4974</td>
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<tr>
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<td>20.5600</td>
<td>2.4882</td>
<td>.6425</td>
</tr>
<tr>
<td>TE_CAD WITH</td>
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<td>17.1667</td>
<td>1.6369</td>
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<tr>
<td>TE_ND WITHOUT</td>
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<td>28.8533</td>
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</table>

3.1 Statistical Analysis: Students’ t’ test showed that the values had a statistically significant difference observed between total etch (T.E) i.e. Adper Single Bond 2 and self etch (S.E) i.e. P 90; caries affected dentin (CAD) and normal dentin (ND); with thermocycling and without thermocycling. (Refer Table 3)

For Adper Single Bond 2 (T.E), in normal dentin the mean values without thermocycling (28.8533) were higher compared to with thermocycling (25.4200). For the same in caries-affected dentin without thermocycling the mean values obtained were higher (20.5600) compared to with thermocycling (17.1667). Without thermocycling, T.E showed higher mean values obtained in normal dentin (28.85) than caries-affected dentin (20.56). With thermocycling, T.E showed higher mean values obtained in normal dentin (25.42) than caries-affected dentin (17.16). Statistically significant difference was not seen, without thermocycling and with thermocycling [p value-0.001]. Without thermocycling, for S.E, the mean values for normal dentin (18.73 MPa) was higher than caries affected dentin, for (15.10 MPa). For normal dentin, without thermocycling total etch (T.E) i.e. Adper Single Bond 2 (28.85) showed higher mean values compared with self etch (S.E) i.e P90 (18.57). (Refer Graph 1)

For caries-affected dentin, without thermocycling, total etch, Adper Single Bond 2 (20.56 MPa) showed higher mean values than self-etch, P90 bonding agent (15.10 MPa). For caries-affected dentin, without thermocycling, total etch, Adper Single Bond 2 (20.56 MPa) showed higher mean values than self-etch, P90 bonding agent (15.10 MPa). Statistically significant results were also ob
For caries-affected dentin, with thermocycling, total etch, Adper Single Bond 2 (17.16MPa) showed higher mean values than self-etch, P90 bonding agent (12.04 MPa). Statistically significant results were also obtained ($p < .000$) (Refer Graph 2) Also, in case of caries-affected dentin without thermocycling the mean values obtained were higher (15.1067) compared to with thermocycling (12.0400).

4. Discussion and Review of Literature
The achievement of high-strength, durable bonds between tooth structure and restorative materials have been a long-term goal of the dental profession. The formation of such bonds would give way to a more conservative, adhesive and esthetic manner of dealing with tooth repair and the universal application of minimally invasive dentistry. Although bond strength testing is done on normal dentin for convenience, clinically most bonding substrates are not in normal dentin, but are in caries affected or sclerotic cervical dentin [15, 16, 18]. Hence, it has been used in comparison with normal dentin in the present study.

However, the structural variations in caries affected dentin may be a challenge to provide effective bonds. Lasting adhesion or the bond strength is the response of an assembly (e.g.: composite-tooth interface) to deformation loads. The development of new adhesive systems aims to create strong adhesion to all types of tooth substrate, particularly caries – affected dentin [15].

When stress is applied to the assembly, energy is absorbed by the adhesive and transferred and distributed to the components of the joint. Failure of such assemblies occurs as a result of flaws, which propagate under stress as cracks and can originate in any component of the assembly [9].

The microtensile bond test was introduced by Sano et al. (Nakajima et al. 1995, Yoshima et al. 1996) and developed to overcome this problem by designing samples to concentrate stress to the bond layer of an assembly [13, 20, 21]. This method has several advantages over other testing methods such as resulting in fewer cohesive failures and producing far more adhesive failures [2, 7, 11, 13, 21].
It also permits appropriate sample size management by the use of only one tooth to fabricate several bonded dentin-resin rods i.e. the number of experimental units (tooth-composite assemblies) \(^9, 14, 20\). Each microtensile specimen is interpreted as a separate experimental unit, regardless of whether it is obtained from the same or different teeth.\(^22\) This provides better reliability of conclusions drawn from the vast literature on adhesives \(^9, 14, 20\).

It allows testing of substrates of clinical significance, such as carious dentin, cervical dentin, and enamel.\(^{14, 20}\) This technique also permits a more uniform stress distribution along the interface between dentin and resin during testing.\(^{11}\) Thus, information about the strength inherent to bonding materials (i.e. adhesives) rather than the adherends (or tooth structure) is generated.

There are also some disadvantages associated with the microtensile test. It is more cumbersome and more difficult to undertake than some of the conventional tests. However, the insights gained are thought to outweigh this issues \(^9\).

Dentin adhesives tend to function well in bond strength tests when tested shortly after application \(^3\). Clinically, the oral environment, with its moisture, physical stresses, changes in temperature and pH, dietary components, and even chewing habits, considerably influences interactions between materials and tooth tissues [Gwinnett, 1990] \(^3, 10\). Although thermal cycling represents only one of these challenges, the other factors may have a detrimental effect on the microtensile bond strength \(^3\).

In our study, the average mean values for Group I (total-etch, Adper Single Bond 2 = 17-28 MPa) had higher bond strength compared to Group II (self etching primer, P90 = 12-18 MPa).

In case of total-etch system, as the demineralised collagen fibril mesh is used as the bonding substrate, a wet bonding technique is required to ensure its full expansion. The ethanol solvent of Single Bond, due to its high vapour pressure, competes with moisture, replacing it and promoting infiltration of monomer through the nano-spaces of the exposed collagen network. This serves as a mesh-work for the creation of a resin-demineralized dentin hybrid layer, resulting in a strong micromechanical interlocking between resin and the superficially demineralized dentin. This could probably account for the higher bond strength values showed by Single Bond compared to other groups \(^20\). This is supported by studies done by Neelima et al. and Yesilurt et al. who found that though the bond strength of AdheSE and Fuji Bond LC was above 30 Mpa, it was less than Single Bond as evaluated by testing of microtensile bond strength \(^{20}\).

The same results were confirmed by Kwong et al., Say EC et al., etc who found that regardless of the conditioning methods, bond strengths to ND were significantly higher than CAD \(^{11, 16, 17, 29}\).

Maria Carolina et al. supported the above results and also found that when bonded to caries-affected dentin, Single Bond had the highest bond strength, followed by Clearfil SE (self etching primer) \(^{15}\). Thus in CAD, the total etch adhesives preformed better than self-etch systems \(^4, 16\). The reason for this seems that caries-affected dentin contains dentinal tubules that are filled with acid-resistant whitlockite minerals that interfere with the infiltration of adhesive reins and the formation of resin tags. The presence of these deposits in the dentinal tubules reduces dentinal permeability to near zero \(^4\). The decrease in modulus of elasticity is also a contributing factor \(^{16, 17}\).

CAD presents lower nanomechanical properties and a lower cohesive strength. Knoop hardness testing of CAD is shown to be half of that of normal dentin. This is because they are dependent on the properties of the intertubular dentin, which is partially demineralised and more porous. The porous nature allows deeper penetration of monomers reflecting in thicker hybrid layers. However the bond strength is unrelated to the hybrid layers. Thus the weakest link is the resin-caries-affected dentin assembly, probably due to the cohesive nature of the CAD \(^4, 16, 18\).

Gwinnett reported that resin infiltration accounted for about one third of the bond strength in acid-etched, sound dentin that was devoid of surface diffusion barriers \(^{11}\).

The application of 37% phosphoric acid, in total-etch, seems to solubilize the intratubular mineral deposits in CAD, better than the weaker acids used in self-etching primers, thereby contributing to better resin retention. Thus the milder acids of the self-etch primers are not acidic enough to dissolve the mineral casts \(^4, 13\). Also; bacteria entrapment within the resin layer may introduce defects that weaken the ultimate strength of the polymerized adhesive \(^{11}\).

In our study, we found that thermocycling reduced the bond strength further of total-etch and self-etch. Bond strengths of CAD were reduced more than ND. This result is supported by authors such as Jan De Munck et al. and Maria Carolina G Erhardt et al.\(^{15, 30}\).

Thermocycling is a widely used artificial aging methodology. The ISO TR 11450 standard indicates that a thermocycling regimen comprising 500 cycles in water between 5 and 55 degree Celsius is an appropriate artificial aging test.

The artificial aging effect induced by thermocycling can be 2-fold i.e. hot water may accelerate hydrolysis of non-protected collagen and extract poorly polymerized resin polymers and due to higher contraction/expansion coefficient of the restorative material (as compared to tooth tissue) repetitive contraction/expansion stresses are generated at tooth-biomaterial interface resulting in cracks that may propagate.
along the bonded interfaces, causing gaps and inwards and outflow of pathogenic fluids called percolation.\textsuperscript{29} CAD bonds are more prone to hydrolytic degradation than ND bonds. According to Wang \textit{et al.}, the most influential character for void formation in CAD is its morphology and environment than increase water content. Even if no water is present during polymerization, environmental water can enter the polymer bulk and trigger chemical polymer degradation. Resin breakdown from hydrolytically unstable polymeric hydrogels within the hybrid layers of CAD may have continued to occur through the nanoleakage channels during aging \textsuperscript{15}.

\textit{In vitro} studies such as the present one, provides an idea of the strength and durability of bond strengths achieved with commonly available dentin bonding agents. However, more clinical research is needed in order to gain insight and further improvise on the present bonding concepts.

5. Conclusion

From the present study it can be concluded that

1. Adper Single Bond 2 (total-etch) had higher bond strength values than P90 (self-etch) in normal dentin as well as caries-affected dentin.
2. Normal dentin showed higher bond strength values compared to caries-affected dentin for both Adper Single Bond 2 and P90 bonding agents.
3. Thermocycling reduced the bond strength of normal dentin as well as caries-affected dentin to both, Adper Single Bond 2 and P90.

These results are obtained within the experimental conditions of the present study. Extrapolation to clinical practice merits further investigations to confirm the observed data. Such studies give us an insight to the durability and longevity of such restorations in the presence of the challenging biomechanics factors of the oral cavity.

6. Acknowledgements

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I would like to claim no benefits either commercial or financial by conducting this study. The study has been conducted in a pure unbiased manner.

7. References

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