Comparative evaluation of microleakage of two self-etching dentin bonding agents on primary and permanent teeth. An in vitro study

ABSTRACT

Aim: Conservative procedures using dentin bonding agents are one of the important aspects of paediatric dental practice. The objective of this in vitro study was to comparatively evaluate the microleakage of two self etching adhesives in primary and permanent teeth. Materials and methods: Sixty-four human anterior teeth (thirty-two primary and thirty-two permanent) were divided into four groups: primary teeth bonded using a 6th generation (Contax) bonding agent; permanent teeth bonded using a 6th generation (Contax) bonding agent; primary teeth bonded using a 7th generation (Clearfil S3) bonding agent; permanent teeth bonded using a 7th generation (Clearfil S3) bonding agent. A Class V cavity was prepared on all samples and were restored with composite resin as per manufacturers’ instruction. After thermocycling, the teeth were stained with methylene blue, sectioned, and measured for microleakage. Results: It was found a statistically significant difference in microleakage between incisal and gingival margins in each of the study group. Conclusion: Clearfil S3 (7th generation) bonding agent could be of greater advantage in paediatric dentistry than Contax (6th generation) because of its fewer steps and lesser microleakage in both primary and permanent teeth.

Keywords: Restorative dentistry/dental materials; Traumatic injuries; Pulp biology; basic sciences.

Introduction

The integrity and durability of the interface between teeth and restorative material is of fundamental importance in dentistry. The acid etch bonding of composite resin to enamel has proven to be an effective method to enhance tooth restoration interface by increasing its strength and decreasing leakage [Van Meerbeek et al., 1996; Swift et al., 2001].

During the past twenty years, significant improvements have often been made in the field of dentin adhesives. Dentin bonding agents used during the early 90s are still in use and are often referred to as a 4th generation of dentin adhesives, that consist of a separate etchant, primer, and a bonding resin. The primer is a hydrophilic monomer dissolved in solvent such as acetone, ethanol or water. The bonding resin consists of the same monomer system used in the composite which consists of three steps (etching, priming, and adhesive placement) needed for placing these adhesives; therefore dentists thought the 4th generation of dentin adhesives complex and time consuming to use, and demanded simpler solutions. The first simplification was the 5th generation of bonding agents, systems in which the primer and the adhesive were mixed together and supplied as a single system. Despite their popularity, the results obtained by these systems did not suggest that they performed as well as 4th generation of dentin adhesives. However, the 5th generation dentin adhesives showed that dentists wanted simpler adhesive systems. Efforts have been made to develop new dentin bonding materials that can withstand the stresses induced by polymerization shrinkage. This has resulted in the evolution of bonding agents with higher bond strengths.

The problems with most currently used adhesive systems are more operative steps, which can lead to clinical errors like moisture contamination and over-desiccation. Such errors can result in premature failure of adhesive restorations. So in an attempt to simplify the clinical procedure some manufacturers combined the etchant, primer, and bonding agent into ‘One - Bottle’ adhesive. As a consequence, systems even simpler to use were developed. Two of such systems evolved, one consisting of an acidic primer and a bonding resin referred to as a 6th generation adhesive, and another in which the etchant, primer and adhesive are combined into one single delivery system marketed as 7th generation of adhesive systems. The advantages of the self-etching system include complete infiltration of the bonding agent into the demineralised dentin and a reduced number of clinical procedural steps. The efficiency of these materials as a bonding agent will be assessed in this in-vitro study [Soderholm et al., 2005; Heping Li et al., 2000].

Materials and methods

Sixty-four sound human anterior teeth, which had been previously extracted, were used in the study; thirty two were primary and thirty two were permanent. The samples were thoroughly cleaned free of debris and calculus using a scaler and water/pumice slurry in a dental prophylactic cup for 20 s and stored in 0.5% chloramine solution for infection control purposes, except for 24 hrs before beginning the experiment, and then they were kept in distilled water. In the test group, the teeth were divided into four groups.

- Group I: 16 primary teeth bonded using a 6th...
generation bonding agent (Contax).

- Group II: 16 permanent teeth bonded using a 6th generation bonding agent (Contax).
- Group III: 16 primary teeth bonded using a 7th generation bonding agent (Clearfil S3).
- Group IV: 16 permanent teeth bonded using a 7th generation bonding agent (Clearfil S3).

One class V cavity was prepared on the labial surface of each tooth used in the study, using a #557 straight fissured diamond bur at high speed handpiece with adequate water cooling. Cavity dimensions were 3 mm wide, 2 mm high and 1.5 mm deep. The occlusal margin was in enamel and the gingival margin was in dentin cementum. The occlusal wall was beveled (45°) with a finishing bur. Each bur was replaced after every 5 cavity preparations. The prepared cavity was rinsed with distilled water and dried with compressed air. The bonding agents were used as per manufacturers’ instructions.

Contax
One drop of Contax prime was dispensed into the mixing pad and was applied to the tooth surface for 20 seconds, following which the surface appeared moist. Then a drop of Contax-Bond was dispensed into the mixing pad and was applied to the primed tooth surface for 20 seconds. The adhesive was thinned out with oil free air, following which the surface appeared moist. Then the adhesive was light-cured for 20 seconds.

Clearfil S3
The necessary amount of Bond was dispensed into a well of the mixing dish, and was applied to the cavity wall and left for 20 seconds. Following conditioning for 20 seconds, the entire adherent surface was dried by blowing high pressure air for more than 5 seconds thus spreading the bond layer into a thin and even film.

Bonding
Then the Bond was light cured for 10 seconds with dental light curing unit. Group I and III were bonded using Contax bonding agent Group II and IV were bonded using Clearfil S3 bonding agent. The preparations were restored with a Z100 composite (3M) in two increments with the Clearfil S3 bonding agent. The preparations were restored in the oral cavity.

Table 1 - Dentin bonding agents used, composition and respective manufacturers.

<table>
<thead>
<tr>
<th>Adhesive system</th>
<th>Manufacturer</th>
<th>Composition</th>
<th>Etchant / primer pH</th>
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<tbody>
<tr>
<td>Contax (6th generation)</td>
<td>(DMG, Hamburg)</td>
<td>Primer (Liquid 1): water, maleic acid, sodium fluoride. 1.3 Bond(Liquid 2): Hydrophilic and acidic BisGMA based resin matrix, catalyst.</td>
<td></td>
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<tr>
<td>Clearfil S3 Bond (7th generation)</td>
<td>(Kuraray)</td>
<td>10 - Methacryloyloxydecyl dihydrogen phosphate (MDP), Bis-GMA, 2-hydroxyethyl methacrylate (HEMA), hydrophobic dimethacrylate, dl-camphoroquinone, ethyl alcohol, water, silanated colloidal silica</td>
<td>2.5</td>
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Microleakage was measured by an operator who was blinded to the type of adhesive. The type of margin (incisal vs gingival), however was evident upon measurement of the image. The image of the enamel-composite interface at the cavity margin was captured at X125 magnification under a stereomicroscope and stored in a digital format. Microleakage, seen as a dark line at the enamel-composite interface was measured in millimeters using Image - pro plus software. Two microleakage measurements were taken for each sample: one for the incisal margin and the other for the cervical margin of the restoration.

The scoring criteria used was as follows.

- 0 - No dye penetration.
- 1 - Dye penetration up to 1/3 of the cavity depth.
- 2 - Dye penetration more than 1/3 but less than 2/3 of the cavity depth.
- 3 - Dye penetration more than 2/3 of the cavity depth.

Results
The degree of marginal leakage was evaluated by the penetration of the dye stain from the cavity margin to the base of the cavity preparation. All the 128 samples were evaluated for dye penetration. Since microleakage was assessed in scores, non-parametric tests were used for analysis. Kruskal-Wallis ANOVA was used for multiple group comparisons and Mann-Whitney test for group wise comparisons.

Statistical software
The Statistical softwares SPSS 11.0 and Systat 8.0 were
undetectable passage of bacterial fluids, molecules or ions between a cavity wall and the restorative material applied to it” [Siddu and Hinderson, 1992].

The current research is focusing on making bonding to enamel a relatively simple process for the users. However, adhesion of composite to dentin is still a universal challenge, because unlike enamel, which is a highly mineralised tissue composed of only 4% organic material, dentin contains 20% organic material and 10% water by weight. This heterogeneous nature of dentin makes bonding always problematic and difficult. Intact mineralised dentin does not permit much monomer diffusion through the tooth structure. Therefore dentin must be suitably conditioned or etched to create channels between collagen fibrils to allow monomers, which have good affinity, to diffuse into the demineralised dentin [Gordan et al., 1998]. After etching it is crucial to maintain the space between the demineralised collagen fibrils following the removal of the hydroxyapatite crystals. This demineralised unsupported collagen matrix can easily collapse causing a decrease in the interfibrillar spacing and loss of permeability to resin monomer [Van Meerbeek et al., 2003]. Hence it is imperative to maintain the structural integrity of demineralised collagen.

Currently employed 5th generation bonding agents require rinsing after completion of the etching process. This rinsing step has to be followed by careful removal of excess water without desiccating the collagen. Despite adequate operator care, most of the times the collagen substrate might either become overly wet or over-dried. In such overly wet conditions excess water that was incompletely removed results in blister and globule formation at the resin/dentin interface [Tay et al., 1996; Tencate, 1989]. Such interface deficiencies undoubtedly weaken the resin/dentin bond. On the other hand, a certain amount of water is recommended to avoid collapse of exposed collagen. Over drying of acid etched dentin surface is thought to induce surface tension stresses, causing the exposed collagen network to collapse, shrink and form a compact coagulate that is impermeable to resin [Pashley and Carvalho, 1997; Paula and Dorothy, 1996]. In order to

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of samples</th>
<th>Microleakage scores</th>
<th>Mean + SD</th>
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</thead>
<tbody>
<tr>
<td>I</td>
<td>16</td>
<td>0 1 2 3</td>
<td>1.1 + 0.9</td>
</tr>
<tr>
<td>II</td>
<td>16</td>
<td>1 1 3 2</td>
<td>0.9 + 0.9</td>
</tr>
<tr>
<td>III</td>
<td>16</td>
<td>1 2 3 4</td>
<td>0.9 + 0.9</td>
</tr>
<tr>
<td>IV</td>
<td>16</td>
<td>1 2 3 4</td>
<td>0.7 + 0.6</td>
</tr>
</tbody>
</table>

Kruskal-Wallis Anova: X² = 2.21 P=0.53 (Non significant)

**TABLE 4** - Comparison of microleakage between occlusal and gingival margins.

**Discussion**

Restoring cervical lesions with resin composites has always been a problem, especially where no enamel is present for bonding to the gingival margin. The higher organic component, tubular structure, fluid pressure and the lower surface energy of dentin make bonding to dentin more difficult than enamel [Pashley and Carvalho, 1997]. Poor adhesion between dentin and restorative material predisposes gap formation. Marginal gap leads to leakage, which may be responsible for secondary caries, marginal discoloration, pulp inflammation and hypersensitivity. [Kidd, 1976; Bauer and Hensen, 1984]. The pattern of microleakage at enamel and dentin is complex. Microleakage has been defined as “The clinical

used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc. For all the tests, a p-value of 0.05 or less was used for statistical significance. Results are reported in Tables 2, 3, 4; Figure 1).

Analysing the data obtained, it was observed that there was no statistical difference in microleakage between 6th and 7th generation dentin bonding systems, when applied onto primary and permanent teeth, however, on inter-comparison between the test groups using Mann-Whitney test it was observed that there was significant differences in the amount of microleakage at the gingival and incisal margins in all groups.

**TABLE 2** - Mean Scores of microleakage at occlusal margin.

**TABLE 3** - Mean scores of microleakage at the gingival margin.
prevent such errors and to simplify the application procedure, self-etching and priming adhesives have been introduced. Self-etching priming adhesives utilize the acidic nature of the primer to demineralise the dentin and simultaneously facilitate the penetration of the resin adhesive [Paula and Dorothy, 1996; Watson et al., 1993]. This system does not require a separate etching, rinsing and drying steps. This eliminates the risk of over etching, over drying and incomplete resin infiltration [Heping Li et al., 2000]. The other major parameter affecting adhesion is the contraction of the restorative composite during the polymerisation resulting in the detachment of the adhesive resin from the underlying hybrid layer [Van Meerbeek and Williams, 1993]. Gaps in free intact surfaces were most frequently observed when a relatively thick layer of a separately polymerized and particle filled adhesive resin is present under the polymerising resin composite. These observations provide evidence for an elastic bonding concept [Zheng et al., 2001; Van Meerbeek and Vargas, 2001], in which a sufficiently thick and relatively elastic unfilled or semfilled adhesive resin may absorb in part the polymerisation shrinkage stresses of the composite material by elastic elongation, thereby preventing the interface from detaching [Van Meerbeek and Williams, 1993]. The adhesion mechanism of self-etching bonding agent is based on smear layer penetration, demineralisation of superficial underlying substrate and monomer diffusion enhancement into the demineralised dentin, facilitating hybrid layer formation.

Self-etching adhesive systems offer 2 advantages in paediatric dentistry.

- Tooth isolation is not always possible with small, sometimes uncooperative and often mouth breathing children. Moreover when etching gel is rinsed, its unpleasant taste often causes a swallowing or a spitting reflex, leading to salivary contamination.
- Etching adhesive systems are a good choice in paediatric dentistry, since the elimination of rinsing and drying steps reduces the possibility of overwetting or overdrying, which can have a negative influence on adhesion.

According to Khera and Chan [1978] the acute angles of cavosurface margin had dramatically more marginal leakage than the middle of the cavity in class V restorations. The use of organic dye as tracer is one of the most common methods for detecting microleakage in vitro. The same was followed in this study and microleakage was evaluated by sectioning the teeth in a labiolingual direction. The present in vitro study compared the bonding effectiveness of two self etching adhesives, Contax and Clearfil S3, using microleakage to evaluate the marginal integrity of restorations in primary and permanent teeth. According to the results of this study, Clearfil S3 exhibited less leakage than Contax, and permanent teeth exhibited less leakage than primary teeth, but were statistically insignificant. However there was significantly less microleakage at the incisal margin than at the gingival margin. These results support the findings of previous studies that have shown no significant difference in microleakage in 6th and 7th generation dentin bonding agent systems in permanent teeth [Deliperi et al., 2007]. Ramin and Astrid [2004] suggested that the probable reason for more microleakage in 6th generation bonding systems could be absence of filler, as the latter helps in forming a thick adhesive layer, so that the ability of the interfaces to maintain adhesion during the critical early stages of polymerisation is better, improving the resistance to dimensional changes. The 7th generation bonding system used in this study contains a high amount of nanofiller, which may promote formation of the adhesive film with appropriate thickness and could also reduce adhesive shrinkage, even if the elasticity modulus and thus the adhesive rigidity will increase. But Schmitt and Lee [2002] in their study concluded that there was no statistically significant difference in microleakage among the filled and unfilled adhesive resins.

Overall ClearfilS3 Bond revealed superior (not necessarily always significant) results at both margin locations. A possible explanation for these results include the type of solvent used. ClearfilS3 utilizes alcohol as the primary component solvent for conditioning dentin surface prior to adhesive component attachment. As these alcohol solvents dehydrate the water filled spaces and dry collagen fibrils, chemically producing a higher monomer to water ratio, stiffening the collagen complex, which is conducive to resin attachment. Moreover ClearfilS3 includes a proprietary “Molecular Dispersion Technology” enabling a two phase liquid, hydrophilic/hydrophobic component homogenous state at the molecular level, reportedly resulting in reduction and/or loss of water droplets at the adhesive interface and therein a superior bond. Also, the 10-Methacryloyloxydecyl dihydrogen phosphate (MDP) adhesive monomer molecular structure allows for decalcification and penetration into tooth structure, “creating a chemical bond to calcium”, whereby MDP chemically bonds to hydroxyapatite, which could be one of the possible reason for less microleakage [Owems and Johnson, 2007].

The other finding in this study was that permanent teeth exhibited less microleakage than primary teeth. Probable reason for this could be the significant differences in the bonding strength and micromorphology between primary and permanent teeth; however, few studies have evaluated the microleakage in primary dentition [Aykroyd et al, 1992]. Salama and Tao [1991] have recommended to reduce the etching time of primary dentin to avoid increasing thickness of the hybrid layer, thus producing a
surface morphology similar to permanent teeth after etching. In this study, the primary and permanent teeth were all restored according to the manufacturers' guidelines which do not specify different etching times for primary and permanent teeth, which could be one of the factors for primary teeth exhibiting more microleakage. Another interesting finding in this study was that the majority of the microleakage was found at the gingival portion of the preparation, which could be due to difference in the quality of the tooth structure between the incisal and gingival aspects of the enamel.

Moreover Avery et al. [1961] determined that the enamel in both human and monkey teeth is harder at the cusp than at the cervical portion of the tooth, while Crabb and Darling [1960] found that the cuspal enamel was more uniformly mineralised than the cervical enamel. Glick [1979] found that the mineralisation begins at the dentin-enamel junction in the cusp of the tooth and then extends in a cervical and peripheral direction such that the surface layer of enamel at the cervical portion of the tooth is the last to be mineralised. Theuns [1983] found a clear gradient in mineralisation from the occlusal to the cervical in premolar teeth, while Wilson and Beynon [1989] confirmed this gradient is present in permanent teeth as well as in primary incisors and canines, but absent in primary molars; nonetheless, this difference in mineral content may account for some of the differences in microleakage between the occlusal and cervical margins in this study. Previous microleakage studies have found significant differences in the amount of microleakage at enamel vs cementum margins. While all margins in this study were in enamel, this further supports the conclusion that mineralisation of enamel towards the gingival aspect has a definitive effect on microleakage.

Conclusions

Within the limitations of this study conducted and results obtained the following can be concluded:

- All adhesives under investigation exhibited a certain amount of microleakage.
- Clearfil S3, a 7th generation adhesive, prevents more microleakage than Contax, a 6th generation dentin bonding agent, used in this study.
- Significant differences were found in the amount of microleakage at the gingival and incisal margins in all groups.
- One-bottle 7th generation dentin bonding systems permit easier application with the same effectiveness as the two bottle 6th generation dentin bonding systems.

References


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