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Introduction

Adhesive dentistry brought profound changes in dental practice. Adhesive techniques combined with the use of aesthetic restorative materials are often required by patients [van Meerbeek et al., 2003]. These requirements, supported by dental professionals, drive the development of new techniques and new materials in dentistry.

Increased aesthetics demand in paediatric dentistry entails the use of adhesive systems that promote satisfactory performance with the lowest number of clinical steps [Perdigão and Lopes, 1999]. For adhesive procedures in primary teeth, simplicity of application is particularly important [Fava et al., 2003].

Currently, dental adhesives can be classified according to the strategy used during the adhesive procedure. Etch-and-rinse adhesives involve separated etch and rinse phases. The acid (30-40% phosphoric acid) is initially applied and rinsed off. This step is followed by a priming step and the application of adhesive resin (3rd step). Simplified two-step etch-and-rinse adhesives combine the primer and adhesive resin into one application [van Meerbeek et al., 2003].

An alternative approach is based on the use of non-rinse acidic monomers that simultaneously etch and prime the dentin: these are the so-called self-etch adhesives. These adhesives are also presented as two-step and one-step or all-in-one systems.

The self-etch adhesive systems were developed in an attempt to create a technique with fewer steps and less operator sensitivity [Perdigão, 2007].

However, some studies found that the effectiveness of self-etch adhesives changes in relation to the etching capacity of the acidic primer and the penetration of monomers into the enamel [Hannig et al., 2003; Miyazaki et al., 2003].

Morphological analysis of the intact enamel/self-etch adhesive interface showed shallow effects [Shimada et al., 2002] and areas without any etching [Torii et al., 2002].

The aprismatic enamel layer of primary teeth is more marked than that observed in permanent teeth [Gwinnett, 1966]. This characteristic interferes with the acid etching pattern, making the adhesion to this substrate worse in comparison with permanent teeth.
Meola and Papaccio [1986] recommend the grinding of primary enamel surface to optimise the adhesive procedure. Few studies have assessed the adhesion to primary teeth structure, since usually adhesion is assessed for permanent teeth [Swift Jr, 2002].

Currently, in view of the preventive techniques and the minimal intervention approach, dental adhesives are becoming fundamental in paediatric dentistry clinic. Quality, stability and longevity are the desired characteristics of the union between the restorative material and the dental substrate. Nevertheless, the performance of adhesive systems applied on primary teeth still lacks extensive research. Consequently, more studies are needed to ensure that quality, stability and longevity can be delivered along with safety, which indicate the success of an aesthetic restoration.

Therefore, this study aims to evaluate the bonding effectiveness of adhesive systems with different adhesion strategies on intact and ground primary dental enamel.

**Materials and methods**

This study was approved by the Research Ethics Committee, School of Dentistry of the University of São Paulo (protocol 192/06).

**Experimental design.** The response variable of this study, bond strength, was quantitatively evaluated by microshear test. The factor variables examined were: enamel surface at 2 levels (intact and ground), and adhesive system at 3 levels (etch-and-rinse, two-step self-etch and one-step self-etch). These sets of variables formed a 2 x 3 factorial array with 10 experimental units per treatment. Sixty sound upper primary incisors, with no fluorosis or hypomineralisation, naturally exfoliated and stored in deionised water under refrigeration (5°C) were randomly divided into six groups according to the type of enamel surface preparation and adhesive system used (Fig. 1).

**Specimen preparation and treatment of the enamel surface.** All teeth were cleaned with slurry of pumice for 20 seconds. The crowns were cut at the cementum-enamel junction (Fig. 2A) using a low speed diamond saw under water-cooling (LABCUT 1010). Thirty enamel surfaces remained intact and 30 middle buccal enamel surfaces were ground flat (Politriz Ecomet 6...
Automet-Buehler) with 400 and 600 grit silicon carbide sandpaper (Buehler). A slight reduction of 0.5 mm deep in the buccal surface was made (Fig. 2b).

All specimens were grounded in palatal faces to obtain slabs 3 mm thick and parallelism between palatal and buccal faces (Fig. 2c, 2d).

**Bonding procedures**

The adhesives were applied on the enamel surfaces strictly following the manufacturers’ instructions (Fig. 3a). Details regarding the selected adhesives systems, such as manufacturer, composition, application technique and batch number, are listed in Table 1.

Prior to light-curing the bonding resin on each specimen, an iris was mounted on each treated surface specimen to restrict the bonding area (Fig. 3b). This iris was cut from microbore Tygon tubing (R-3603, Norton Performance Plastic) with internal diameter and height of approximately 0.8 and 0.5 mm, respectively. After light curing (Jetlite 4000 Plus, J. Morita) for 10 seconds, a microhybrid composite (shade A3, Filtek Z250, 3M ESPE) was carefully inserted into the tubing lumens. Moreover, a clear cellophane sheet was placed over the resin and gently pressed. The resin was then light-cured for 40 seconds (Figure 3C) [Shimada et al., 2002; de Carvalho et al., 2008; Pivetta et al., 2008]. The specimen were stored in deionised water at 37°C and the Tygon tubings around the composite cylinders were removed after 1 hour by gently cutting the tube using a feather blade. All specimens were stored in deionised water at 37°C for 24h (Fig. 3d).

**Micro shear strength bond testing (µSBS testing)**

After storage time the specimens were adhered with cyanoacrilate adhesive (Loctite 454, Henkel Loctite Corp.) to the test apparatus, which was placed in a universal testing machine (Mini Instron 4442, Canton) for microshear bond testing. A thin steel wire (diameter 0.20 mm) was looped between the load cell projection and the resin cylinder, making contact through its lower half circumference. The thin steel wire was gently held flush against the enamel/resin interface (Fig. 3e). The shear force was applied at 1 mm/min crosshead speed until the fracture occurred [Shimada et al., 2002; de Carvalhal et al., 2008]. The µSBS was expressed in MPa as derived from the division of the imposed force (N) at the time of fracture by the bond area (mm²).

**Statistical analysis**

Statistical analysis showed that the sample distribution was normal and homogeneous, allowing the use of a parametric test. Data were analysed with the two-way ANOVA parametric test using a factorial design with the adhesive approach and substrate type.

### Table 1 - Chemical composition and application method of the adhesives tested.

<table>
<thead>
<tr>
<th>Product name (manufacturer)</th>
<th>*Composition (batch no.)</th>
<th>Classification</th>
<th>Application</th>
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<tbody>
<tr>
<td>Adper Single Bond 2 (3M ESPE, St. Paul, Minn, USA)</td>
<td>Etchant: 37% Phosphoric acid, silica thickener HEMA, water, ethanol, amines, Bis-GMA, methacrylate-functional, copolymer of polyacrylic and polyitaconic acids, dimethacrylates, spherical silica particles (6HL.2009-06)</td>
<td>Two-step etch-and-rinse</td>
<td>H3PO4 conditioning 15 s. Rinse with water spray 10 s and dry 5 s. Apply two consecutive coats of adhesive. Dry gently for 5 s. Light-cure for 10 s.</td>
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<tr>
<td>Clearfil SE Bond (Kuraray Co, Osaka, Japan)</td>
<td>Primer: 10-MDP, HEMA, hydrophilic dimethacrylate, photo initiator, water (00480A); Bond: 10-MDP, Bis-GMA, HEMA, hydrophilic dimethacrylate, microfiller (00666A)</td>
<td>Two-step self-etch</td>
<td>Apply the primer for 20 s; gently air-blow; apply the bond and light-cure for 10 s.</td>
</tr>
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</table>

*Composition as provided by the manufacturers: Bis-GMA, bisphenol-glycidyl methacrylate; DM, dimethacrylate; GPDMS, glycerol phosphate dimethacrylate; GDM, glycerol dimethacrylate; HEMA, hydroxyethylmethacrylate; 10-MDP, 10-methacryloyloxydecyl dihydrogen phosphate; PAMM, phthalic acid monoethyl methacrylate; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate; MAC-10.
as variables. Tukey’s HSD multiple comparisons statistical test at a 0.05 significance level was used.

**Scanning Electron Microscopy (SEM)**

In order to observe the effect of the conditioning with phosphoric acid etching and self-etching primers on the ground and intact enamel surfaces, six primary enamel surfaces were prepared and treated with etching materials according to the manufacturers’ instructions. The enamel surfaces etched with self-etch primer were rinsed with acetone for 10 seconds [Nikaido et al., 1997] to remove the self-etch primer. Then, the specimens were dehydrated in increasing concentrations of ethanol and water up to 100% ethanol. Specimens were gold sputter-coated (Sputter Balzers SCD 040) and observed under SEM (LEO 440 Scanning Electron Microscope, Leica Microsystems Vertrieb GmbH) at 10kV of accelerating voltage. The working distance was kept at 10 mm and the magnification was standardised at X2000.

**Results**

**Microshear bond strength testing**

The mean and standard deviations of µSBS are shown in Table 2. Two-way ANOVA indicated significant effects for enamel surfaces (ground and intact) (p=0.005), adhesive systems (p<0.0001) and for interaction between them (p=0.002).

Considering the enamel surface factor, only two-step self-etch showed higher µSBS values when the enamel was grounded.

**Etch-and-rinse (SB) adhesive** showed higher µSBS values compared with self-etch adhesive systems (SE and OBF), which demonstrate similar performance within intact enamel groups.

The two-step self-etch adhesive (SE) was statistically superior to etch-and-rinse (SB) and to one-step self-etch (OBF) adhesives when enamel was grounded. One-step self-etch (OBF) showed the lowest µSBS values.

**Morphological observation by scanning electron microscopy (SEM)**

The demineralisation pattern varied according to the surface and etching system used. Morphological
The primary enamel morphology of intact and ground surfaces were changed after the treatment with phosphoric acid and showed similar etching patterns (Fig. 4A, 4B). The smear layer was completely removed from the ground enamel surface. Dissolution of prism cores and boundary regions can be observed in both substrates.

The intact enamel surface was predominantly unetched by two-step self-etch primer (Fig. 4C), while ground enamel surface revealed moderate and shallower demineralisation compared to phosphoric acid pattern (Fig. 4D).

Intact and ground primary enamel surfaces treated with one-step self-etch primer showed preponderantly no demineralisation. Ground enamel was slightly changed when compared to intact enamel (Fig 3E, 3F).

Discussion

Adhesive procedures in primary enamel are commonly considered complex processes, as some studies conclude that adhesion to this substrate is not as reliable as to permanent enamel [el Kalla and Garcia-Godoy, 1998; Olmez et al., 1998].

The application of adhesive systems to primary dental tissue is a delicate procedure [Finger and Fritz, 1996] if carried out in laboratory studies as well as in clinical routine, due to the size and anatomy of the tooth. Therefore, such procedure requires a range of additional cares.

Choosing an adhesive system in paediatric dentistry depends not only on its bond strength characteristics. The selection also needs to take into account patients’ behavior and age. For children with difficulty in accepting dental treatment, the procedures must be fast. Thus, in such cases, the use of an adhesive with fewer operative steps [Atash and van den Abbeele, 2005] and less sensitive technique is essential. Consequently, the use of adhesive systems with self-etch primers would be helpful for such cases as it simplifies the bonding process by reducing the number of clinical steps.

The primary enamel presents different surface characteristics depending on the dental pathology presented. In cases of carious teeth with injuries or fractures, the primary enamel has its surface grounded before the restorative procedure. However, in cases of aesthetic and functional repair of teeth with morphological changes (e.g. conoid teeth) or bracket bonding, primary enamel can be submitted to the adhesive procedure without preparation. From a clinical perspective, cavity preparation often includes intact and ground enamel [Di Hipólito et al., 2005]. Therefore, it is necessary to evaluate the performance of different adhesive systems on intact and on ground primary enamel.

Garcia-Godoy and Gwinnett [1991] described as “coral-like”, the appearance of unground enamel that traditionally was called aprismatic layer or “prismless” enamel as, in such substrate, the presence of prisms is factual despite the fact that their orientation under
SEM is different. The presence of this “coral-like” enamel must be considered when bonding on intact primary enamel, due to its acid etching resistance characteristic [Garcia-Godoy and Gwinnett, 1991; Kanemura et al., 1999; Pashley and Tay, 2001]. Grinding the enamel before etching produced a uniform distribution of prismatic structure. If the enamel surface was not ground, a “coral-like” porous retentive surface was apparent in most of the cases with no defined prismatic structure [Garcia-Godoy and Gwinnett, 1991].

Etch-and-rinse adhesive system (SB) performed alike in both substrates evaluated. Similarly to other studies, it was observed that 37% phosphoric acid application created comparable etching patterns [Gwinnett and Garcia-Godoy, 1992; Garcia-Godoy and Gwinnett, 1999; Hannig et al., 2003; Shinohara et al., 2006; Pivetta et al., 2008], providing analogous resin penetration into ground and intact enamel.

Other studies confirm the findings of this experiment that suggests that self-etch adhesive systems are more effective when used on ground enamel compared to intact enamel [Kanemura et al., 1999; Shinohara et al., 2006].

The influence of permanent enamel surface grounding on bond strength of adhesive systems was evaluated by Senawongse et al. (2004). The authors reported that the enamel surface preparation prior application of one-step and two-step self-etch adhesives (OBF and SE respectively) resulted in higher bond strength values than those obtained on intact enamel surfaces.

Pashley and Tay (2001) proposed a self-etch adhesives classification according to the pH of their primers. Adhesives systems with different pH can produce different etching patterns of enamel, given their acidity differences. Based on these characteristics, the self-etch adhesives can be classified as strong and mild, considering strong those with pH ≤1, which produces enamel demineralisation similar to phosphoric acid etching pattern. The so-called mild have pH >2 and do not have the same demineralisation capacity of phosphoric acid.

Previous studies showed that the enamel etching degree depends on the self-etching primer pH [Kanemura et al., 1999; Pashley and Tay, 2001; Hannig et al., 2002; Pivetta et al., 2008]. Commonly, a lower pH provides a higher enamel etching capacity to the self-etchant than a mild pH [Grégoire and Ahmed, 2007]. The present study demonstrated that the etching agent acidity influenced in the bond strength values and in the demineralization patterns of intact and ground enamel.

One-step self-etch adhesive system showed the lowest bond strength values independently of the substrate used (intact or ground enamel). This result can be probably attributed to the higher acidity and lower aggressiveness of its acidic monomer (pH = 2.9) when compared to the two-step self-etch adhesive system (pH = 1.9) [Inoue et al., 2005].

The SEM is an effective analytical way to assess the performance of adhesives on the superficially demineralised enamel substrate [Grégoire and Ahmed, 2007]. The electron micrographs showed a divergence in adhesives systems etching capacity used. These differences are related to the primer acidity. Through another microscopic morphological investigations, it was also observed that enamel etching capacity depends on self-etch primers pH [Kanemura et al., 1999; Pashley and Tay, 2001; Hannig et al., 2002].

The results demonstrated that only the two-step self-etch adhesive showed higher bond strength values when applied on ground enamel. This result may be justified by the composition of this adhesive system. Its functional monomer 10-MDP (10-methacryloxydecyl dihydrogen phosphate) is characterised by higher capacity to establish linkage to hydroxyapatite and the stability of these linkages when compared to other self-etch adhesive functional monomers [Inoue et al., 2005; Yoshida et al., 2004]. This chemical interaction of acidic monomers with enamel justifies the good performance of the two-step self-etch, turning it into the “gold standard”, against which the others self-etch systems should be compared to.

Increased demand for aesthetic restorations in paediatric dentistry boosts the search for more suitable restorative materials and, therefore, the investigation of materials able to simultaneously reduce the operative time without compromising the quality of the work [Torres et al., 2007].

Conclusion

Considering the performance of the adhesive systems evaluated in this study, and the need to simplify clinical procedures in paediatric dentistry, the use of two-step self-etch adhesive systems becomes a reliable alternative to the use of etch-and-rinse adhesive systems on both ground and intact primary enamel.

References


Di Hipólito V, de Goes MF, Carrilho MR, Chan DC, Daronch M, Sinhoreti MA. SEM evaluation of contemporary self-etching