Evaluation of three different adhesive systems using a bacterial method to develop secondary caries in vitro

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ABSTRACT: Purpose: To assess the effects of three different dental adhesive systems on the formation of secondary root caries, in vitro, with a standardized interfacial gap in a filled cavity model. Methods: 40 sound human molars were selected and randomly assigned to four experimental groups: Clearfil SE Bond (CSEB), Xeno III (X-III), Scotchbond Multi-Purpose Plus (SBMP) and negative control (NC) without an adhesive system. After the standardized Class V cavity preparations on the buccal and lingual surfaces, restorations were placed with resin composite (Filtek Z250) using a standardized interfacial gap, using a 3 x 2 mm piece of 50 µm metal matrix. The teeth were sterilized with gamma irradiation and exposed to a cariogenic challenge using a bacterial system with Streptococcus mutans. Depth and extension of wall lesions formed and the depth of outer lesions were measured by software coupled with light microscopy. Results: For wall lesion extension the ANOVA test showed differences between groups except between X-III and SBMP (P=0.294). The Tukey’s test of confidence intervals indicated smaller values for the CSEB group than for the others. For wall lesion depth the CSEB group also presented the smallest mean values of wall lesion depth when compared to the others (P≤0.0001) for all comparisons using Tukey’s test. Regarding outer lesion depth, all adhesives showed statistically similar behavior. SEM evaluation of the morphologic appearance of caries lesions confirmed the statistical results showing small caries lesion development for cavities restored with CSEB adhesive system, which may suggest that this adhesive system interdiffusion zone promoted a good interaction with subjacent dentin protecting the dental tissues from recurrent caries. (Am J Dent 2010;23:93-97).

CLINICAL SIGNIFICANCE: The interdiffusion zone formed by self-etch Clearfil SE Bond adhesive system and subjacent dentin may protect dental tissues facing a secondary caries attacks.

Introduction

Secondary caries is a major reason for the replacement of restorations and this fact has prompted the development of materials, such as dental adhesive systems, with protective properties against caries. The primary aim of dental adhesives is to provide retention for composite restorations, but adhesives should also be able to prevent leakage along restoration margins by adequate cavity sealing and antibacterial action.

Dental adhesive systems usually present two ways of conditioning the dental tissues. The etch-and-rinse system removes the smear layer and demineralizes the subsurface dentin and enamel using a strong acid, commonly phosphoric acid. The self-etching system dissolves and incorporates the smear layer into the hybrid layer using acidic monomers and organic acid. Luz et al showed that both systems form an interdiffusion zone, each one with different characteristics of resin tag formations, hybrid layer thickness and degree of adhesive penetration.

Insufficient resin impregnation to form the hybrid layer, besides weakening the bonded interface, may reduce the protection against caries. Some studies reported that in the etch-and-rinse adhesive systems this phenomenon is more evident due to possible discrepancy between dentin demineralization and adhesive impregnation along the resin-dentin interface.

In order to overcome this problem, self-etching adhesive systems were developed in an effort to simplify bonding procedures. In this system, the etched substrate is fully infiltrated by adhesive, due to specific monomers, which simultaneously demineralize and infiltrate the dentin. The self-etch strategy became a less sensitive system because it eliminated the critical wet bonding technique.

The effects of several restorative materials have been studied with models that simulate caries challenge. However, the effect of different adhesive systems using a standardized interfacial gap in a filled cavity model is not well known. The artificial gap ensures bacterial development in the cavity wall and restorative material interface. Although the cavity wall is covered with bonding agent, it is possible to analyze the level of protection of each adhesive system against secondary caries.

This study evaluated the hypothesis that a bonded interdiffusion zone produced by different dental adhesive systems protected dentin at different levels against a caries challenge using a bacterial method and an interfacial gap in a filled cavity model, in vitro. The null hypothesis was that the different bonding systems studied provided similar protection to subjacent dentin facing a caries challenge, within the limitations of this study.

Materials and Methods

Forty extracted, non-erupted, human third molars without previous lesions or visible enamel defects were stored in distilled water at 4°C. Approval by the Ethics Committee, School of Dentistry, University of São Paulo (FOUSP, protocol 58/06) was obtained.

After the apical 2/3 of the roots were removed, the teeth were sectioned with double face diamond discs (Discoflex®) in two fragments: buccal and lingual. The dental fragments were considered independent.

Standard Class V preparations, 2 mm deep, 5 mm long and 2 mm wide (the occlusal margin in enamel and the gingi-
Table 1. Characteristics of the adhesive systems used.

<table>
<thead>
<tr>
<th>Adhesive systems</th>
<th>Composition</th>
<th>pH</th>
<th>Use mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearfil SE Bond</td>
<td>Self-etch primer: MDP, hydrophilic dimethacrylate, HEMA, water</td>
<td>2.0</td>
<td>Primer applied for 20 seconds (no mixing required), bond applied and light-cured for 10 seconds.</td>
</tr>
<tr>
<td></td>
<td>Adhesive: MDP, hydrophilic aliphatic dimethacrylate, HEMA, HEMA,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>bis-GMA, silanated silica</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xeno III</td>
<td>Bottle A: HEMA, ethanol, water, aerosol, stabilizers</td>
<td>&lt;1</td>
<td>Equal amounts of liquid A and B dispensed and mixed for 5 seconds. Applied on dentin and left undisturbed for 20 seconds. Light-cured for 10 seconds.</td>
</tr>
<tr>
<td></td>
<td>Bottle B: Pyro-EMA, PEM-F, UDMA, CQ, BHT, ethyl-4-dimethyl aminobenzoate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adper Scotchbond</td>
<td>Etchant: 35% phosphoric acid</td>
<td>3.3</td>
<td>Dentin etched for 10 seconds, washed and rinsed; primed and dried with gentle air stream. Bond applied and light-cured for 10 seconds.</td>
</tr>
<tr>
<td>Multi-Purpose</td>
<td>Primer: HEMA, polyalkenoic acid polymer, water</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adhesive: Bis-GMA, HEMA, tertiary amines, photo-initiator</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Diagram showing: A. Dental fragment with a Class V cavity and metal spacer on the gingival wall to create an interfacial gap; B. The traced lines show the sections in a buccolingual direction along the restoration extension; C. With filled cavity and interfacial gap, lateral view of one section.

Fig. 2. Measurements (variables) used: wall lesion depth (WD); wall lesion extension (WE); outer lesion depth (OD). R: restoration; G: gap; D: dentin.

val margin in dentin) were made on dental fragment surfaces with a cylindrical plain cut diamond bur in a high speed air turbine handpiece under water spray.

The dental fragments were randomly divided into four groups (n=20), each one restored with one of the following dental adhesive systems: CSEB: Clearfil SE Bond,5 a self-etching adhesive system; X-III: Xeno III,6 a self-etching adhesive system; SBMP: Scotchbond Multi-Purpose Plus,6 an etch and rinse adhesive system; NC: negative control, without adhesive system. Following cavity preparation, the teeth were cleaned with water slurry of pumice and anionic detergent. The application of the adhesives followed the manufacturers’ instructions. Information about characteristics of the adhesives is summarized in Table 1.

After the adhesive system application, except in the control group, the gingival wall was covered with a metal spacer with 50 μm, measured with a digital micrometer, to create a standardized interfacial gap10,13 (Fig. 1A). Then, all experimental cavities were filled with a resin composite (Filtek Z2507), including the control group. After 24-hour irradiation (25 kGy). Then the metal spacer was carefully removed from the cavities.

Steel wires were attached to each dental fragment and the surfaces were painted with acid-resistant nail varnish, except on the restorations and on a 1 mm-wide border around them. The dental fragment/wire units were sterilized with gamma irradiation (25 kGy). Then the metal spacer was carefully removed from the cavities.

The development of carious lesions was induced, in vitro, with a bacterial system, following the method used by others4,15 and modified by Gama-Teixeira et al.11 The microorganism used was Streptococcus mutans (ATCC 25175), which was incubated in TSB8 with 5% sucrose (37°C for 24 hours) in order to induce bacterial growth. The bacteria was placed in a solid culture medium TSA8 to obtain isolated colonies and was re-incubated (37°C for 48 hours). Then, the grown colonies were transferred to tubes containing TSB and 5% sucrose, start-
lesions, ANOVA followed by Tukey’s test was used for pairwise comparisons between groups; P values below 0.05 were considered significant in all comparisons. By the lack of equality of variances, Brown and Forsythe’s test was used to analyze the extension of wall lesions, followed by all pairwise comparisons between groups with the significance limit set and adjusted at 0.0083. This limit corresponds to the division of the level 0.05 by the number of pairwise comparisons considered and it is known as the Bonferroni procedure.16

The same three sections of each experimental group used for the measurements were examined using scanning electron microscopy (SEM), in order to visualize the morphologic characteristics of the lesions.

Results

Macroscopic analysis - After the first 24-hour incubation period, the culture media became turbid, and after 48 hours a thin layer of a dental plaque-like substance covered the tooth fragment surfaces, varnished and unvarnished. This covering substance continuously grew thicker during the 30 days of incubation. Macroscopically, outer lesions in dentin were observed with a softened and yellowish aspect, with different shapes according to the adhesive systems.

Histological features of lesions - Microscopically, artificial caries lesions developed adjacent to the restorations for all materials, with different shapes according to the adhesive systems, and it was possible to define the two zones of secondary caries, a zone on the tooth surface (outer lesion) and another along the cavity wall (wall lesion).17 The wall lesion was characterized when the inner border of an outer lesion curved downward to meet the cavity wall.

A histological evaluation of the lesions showed a surface layer more mineralized above the lesion body, which had a rectangular shape, and sometimes a sclerotic zone was present below the lesion body due to caries crystals deposition into the dentin tubules18 (Fig. 3).

Statistical results

Outer lesions - To analyze the outer lesion depth (OD) ANOVA was used and detected differences between groups (P ≥ 0.004). Tukey’s test with confidence interval at 95% showed that there were significant differences between groups only when the adhesives were compared with NC, except for X-III group, which showed mean values of outer lesion depth larger than the other groups (Table 2).

Wall lesions - For the wall lesion extension (WE) the Brown and Forsythe test showed significant differences between groups (P < 0.001). Then, to detect these differences, pairwise comparisons were used with the same test. The results showed that there were significant differences between all groups, except between X-III and SBMP (P = 0.294) (Table 2).

For wall lesion depth (WD), ANOVA was used (P < 0.001) followed by Tukey’s test with a confidence interval at 95% (Table 2). This analysis showed no significant differences in

Table 2. Means, standard deviations (SD) and confidence intervals (CI) for all variables studied.

<table>
<thead>
<tr>
<th>Group</th>
<th>Wall lesion depth</th>
<th>Wall lesion extension</th>
<th>Outer lesion depth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (mm) CI*</td>
<td>Mean (mm) CI*</td>
<td>Mean (mm) CI*</td>
</tr>
<tr>
<td>CSEB</td>
<td>0.162 ± 0.320 0.01; 0.31</td>
<td>0.013 ± 0.022 0.00; 0.02</td>
<td>0.280 ± 0.100 0.23; 0.33</td>
</tr>
<tr>
<td>X-III</td>
<td>1.090 ± 0.272 a 0.96; 1.22</td>
<td>0.172 ± 0.058 b 0.14; 0.20</td>
<td>0.315 ± 0.054 0.29; 0.34</td>
</tr>
<tr>
<td>SBMP</td>
<td>0.967 ± 0.531 a 0.71; 1.22</td>
<td>0.152 ± 0.078 b 0.12; 0.19</td>
<td>0.308 ± 0.071 0.27; 0.34</td>
</tr>
<tr>
<td>NC</td>
<td>0.990 ± 0.393 a 0.80; 1.18</td>
<td>0.252 ± 0.091 0.21; 0.30</td>
<td>0.391 ± 0.105 0.34; 0.44</td>
</tr>
</tbody>
</table>

Data is displayed as Mean ± standard deviation and CI* (Confidence Interval at 95%). n=17 for CSEB and SBMP groups, n=16 for X-III and NC groups. Superscript letters show equal mean values.
depths of wall lesions between SBMP and X-III groups (P= 0.804), SBMP and NC groups (P= 0.998) and X-III and NC groups (P= 0.889). The CSEB group showed the lowest mean values when compared to the other groups (P< 0.0001 for SBMP, X-III and NC comparisons). These differences are indicated in the confidence intervals shown in Table 2.

SEM evaluation - Results from morphologic evaluations by MEV suggest that the CSEB group maintained the best interaction with subjacent dentin after the caries process when compared with X-III and SBMP groups that may be reduced by the different degree of dentin disorganization facing the caries challenge. The dentin structure below the caries lesion in the CSEB group was less disorganized than in the SBMP and X-III groups (Fig. 4).

Discussion

The bacterial method used for developing caries-like lesions is frequently used because it allows the formation of in vitro lesions similar to in vivo conditions, to evaluate the behavior of restorative dental materials facing a bacterial challenge, although excluding the remineralization periods.

Streptococcus mutans is considered one of the most important microorganisms in the etiology of caries and has been used in many caries studies. In this study, the demineralization on the outer surfaces appeared to be caused by acid production from Streptococcus mutans, which colonized the tooth surface, while the demineralization along the cavity wall was caused by a combination of primary acid attack from the outer dental plaque and additional acid attacks from bacteria which colonized inside the artificial gap between the gingival bonded cavity wall and the restoration.

The aim of the artificial gap was to standardize a gap formation in order to enhance bacterial infiltration, creating a propitious environment for their growth as gaps in adhesive restorations are common when the adhesive resins are not well applied or when contraction stress of the resin restorative material is higher than the bond strength to dentin. In this study, although the gap was created between adhesive resin and restorative resin, if the interdiffusion zone was not well established it was supposed that caries lesion will develop easier, in other words it was supposed that the adhesive resin may protect the dentin against a caries challenge depending on its interaction with the dentin also on its antibacterial properties. As the antibacterial properties of the adhesive systems were not the main focus of this experiment, it was just related with the results achieved here. The size of the gap between restorations and tooth structures was evaluated and shown to be similar to all experimental groups. The presence of a channel for microleakage was a necessary condition for the formation of wall lesions. This is sometimes impossible by using only the thermal cycling method although this was not the only contributing factor.

The outer lesion depth was not affected by the adhesive system, as expected, probably because they do not present antibacterial properties able to produce an inhibition zone facing a caries challenge around the restoration as it occurs with fluoride release from glass-ionomer cement restorations. The shape of lesions varied; probably due to enhanced dental plaque formation in some adhesives, as it commonly occurs in vivo on smooth dental surfaces.

The caries lesion along the cavity wall was influenced by the adhesives used. The materials studied were different regarding their application techniques, etched system, composition, pH, viscosity and fluoride releasing, characteristics that may have modified the results.

Concerning the acidity of the primer, regarding etch-and-rinse for self-etching dental adhesives, their low pH is able to eliminate residual bacteria from the cavity walls and confer antibacterial properties to the adhesive system. Protection of dental-pulp complex and antibacterial activity are desired properties of restorative materials, especially for self-etching adhesives, which partially remove the smear layer and residual bacteria may remain on cavity walls at the interface, increasing the risk of secondary caries, although this rarely occurs. Therefore, high aggressiveness of the conditioner used, as well as proprieties of the functional monomers, must co-determine the bond quality and consequently determine its behavior facing a bacterial challenge. However, De Munck et al considered that some two-step self-etch systems, such as CSEB, may establish good chemical bonds between specific compounds and residual hydroxyapatite crystals enhancing bond stability. Unprotected and partially resin-coated collagen fibrils are thought to be more prone to hydrolysis or enzymatic attacks and other sources of stress. The interfacial degradation is expected to begin on the demineralized dentin zone that is insufficiently infiltrated with resin and occurs especially at the bottom of the hybrid layer produced by etch-and-rinse systems and high acidic monomers.

The acidity of the X-III primer used in the present study (pH <1), makes it a strong self-etching adhesive system, with a rather deep demineralization similar to phosphoric acid etching. With this adhesive, probably the collagen fibrils were partially resin-enveloped and could have been easily degraded with bacterial acid attack as shown by a larger extension of the wall lesion in the X-III and SBMP groups, compared to the CSEB group, and confirmed by the SEM images.

Some manufacturers have produced specific monomers, such as 10-MDP, which is able to form strong ionic bonds with calcium, and is effective for bonding to enamel and/or dentin hydroxyapatite. In agreement with Keshima et al, the CSEB showed the best results concerning the size of secondary caries lesions that corresponded to the most upright interdiffusion zone of dentin and dental adhesive below the lesions.

Fluoride released by glass-ionomer cements prevents caries. The aim of PEM-F, Xeno III’s monomer is to release fluoride, conferring protection for dentin against acid attack. However, the role of fluoride in adhesives is uncertain because this compound is blended with a hybrid layer after light-curing the dental adhesives and it is present only in low concentrations inside this material. In this study, the fluoride present in X-III was not capable of inhibiting secondary caries. The fluoride confined in the polymerized adhesive resin matrix may not be able to be released into the environment.

The adhesive technology has been simplified providing good interaction with tooth tissues, besides reducing postoperative sensitivity. However, recent findings have revealed that most simplified systems were shown to be less durable, while three-step etch-and-rinse and two-step self-etch adhesives show...
better long-term performances.\textsuperscript{6,34} The combination of hydrophilic and hydrophobic monomers in one-step blended adhesives requires a high concentration of solvents to keep substances in the solution, which may harm the behavior of the dental adhesive system.\textsuperscript{7,35} The low performance of X-III should be attributed in part to this factor. The mixture of compounds of X-III made the hydrophilicity high and the adhesive layer behavior as a permeable membrane highly susceptible to hydrolysis.\textsuperscript{35}

The present study concluded that the two-step mild pH self-etching CSEB system showed the smallest wall caries lesions and better interaction with the subjacent dentin than the two-step moderate pH etch-and-rinse SBMP system and the one-step aggressive pH self-etching X-III system, providing some protection against bacterial caries attack. This could be attributed to the intimate interdiffusion zone formed with subjacent dental tissue. However, more studies are necessary to evaluate the role of adhesives in the prevention of secondary caries development.

a. KG Sorensen, Barueri, SP, Brazil.


c. Dentply, Konstanz, Germany.

d. 3M ESPE, St. Paul, MN, USA.

e. Difco-Becton, Dickinson and Company-Sparks, Detroit, MI, USA.

f. Redlease, Araçatiguama, SP, Brazil.

g. Carl Zeiss, Thornwood, NY, USA.

h. Softium, Fortaleza, CE, Brazil.

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References


