The effect of long-term storage on the microleakage of composite resin restorations – qualitative and quantitative evaluation†

Influência do tempo de armazenamento na microinfiltração de restaurações de resina composta – avaliação qualitativa e quantitativa

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ABSTRACT: The aim of this study was to evaluate the influence of storage periods of 24 hours and 3 months on the microleakage of class II cavities. Two methods of assessing microleakage were also compared. Class II cavities were prepared in sound human molars. MO cavities were restored using ABF experimental (Kuraray Medical Inc.) + Z250 composite resin (3M ESPE), and DO cavities were restored using Single Bond (3M ESPE) + Z250. After different storage periods, specimens were thermocycled, immersed in a dye (0.5% methylene blue solution for 4 h) and longitudinally sectioned. Dye penetration was scored according to a 0-4 scale. The extent of microleakage was measured using the ImageLab 2000 program. A statistically significant correlation was verified between both evaluation methods (r = 0.978, p < 0.001). ANOVA revealed a statistically significant difference between the tested adhesive systems regarding microleakage (p < 0.001), although it was not influenced by the different storage periods.

DESCRIPTORS: Dentin-bonding agents; Dental leakage; Dental materials.

INTRODUCTION

The rapid pace at which adhesive systems and aesthetic restorative materials are developed and introduced in the marketplace has contributed to enhance the quality of restorative dentistry over the last few years. Although this fact is uncontestable, adhesive bonding to dental structures is still a great challenge, specially to areas in dentin.5,9,15,18,26 Failures in the tooth/restoration interface may result in bacterial penetration or infiltration of saliva components, which may result in marginal discoloration, secondary caries and even pulp injury.5,19,20,27

In an attempt to minimize such problems, manufacturers have introduced new products with different formulations and more effective techniques. Nowadays, the most commonly used adhesive systems try to combine effectiveness with simplicity, so that they can be used both in dentin and enamel to provide a reliable and long-lasting bond to all types of dental materials.

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Among these, one-bottle and self-etching primers must be emphasized. One-bottle systems combine priming and bonding components in a single bottle, and must be applied to moist dentinal substrate, previously etched using phosphoric acid. Leaving dentin moist renders the technique sensitive, since it is difficult to clinically measure the ideal level of moisture that must be left following washing of the etching agent. Overdrying may result in the collapse of collagen fibers and prevent adequate monomer penetration. On the other hand, excessive moisture may promote dissolution of bonding monomers and damage the adhesive process. These difficulties may result in post-operative sensitivity, which is generally caused by an imperfect sealing of the dentinal tubules after restoration completion.

Self-etching primer systems were then developed in an effort to minimize technique sensitivity. They were recently introduced in the Brazilian marketplace and are based on the use of an acidic monomer which promotes decalcification of the mineral components and does not require washing and drying steps. The penetration of hydrophilic monomers throughout dental structures occurs simultaneously with tissue demineralization followed by application of the bonding resin. Clinically, besides combining etching and priming steps, this system eliminates the need for standardization of the moisture that should be left in a specific level before primer application. Moreover, the risk of incomplete impregnation of the demineralized dentin layer with the priming agent is also eliminated, as demineralization occurs at the same depth as the acidic monomers penetrate, since they are combined. Hence, an enhanced dentinal sealing is theoretically guaranteed, thus reducing the probability of postoperative sensitivity.

Both one-bottle and self-etching primer systems have been constantly studied and due to the great development of new products, laboratory research becomes of fundamental importance, since they try to predict the clinical performance of a new material in a short period of time. However, most studies performed these assays 24 hours after the restoration was finished, without considering a possible marginal degradation of the tooth/restoration interface in such a hostile moist environment as the oral cavity. Undoubtedly, it is also of great importance to evaluate the performance of new adhesive systems following a certain period of time.

Among laboratory tests, we have to emphasize the evaluation of marginal sealing, which is performed using several methods. The analysis of microleakage by means of dye penetration is one of the most commonly used methods, due to its simplicity and quick accomplishment. Traditionally, microleakage assessment is performed by visual analysis, performed by previously calibrated examiners. Although this is the most commonly employed method, dye penetration analysis may be subjective and result in less precise results, even after examiner calibration.

Recently, the morphometric evaluation of microleakage has been used in an attempt to reduce the subjectivity of visual methods, as it is considered as more reliable, although it requires special equipment. In this method, measurements are performed on the linear extension or on the area of microleakage with the aid of an image processor software. Specimens are photographed and, after digitalization, the software delimitates dye penetration using a special tool from the referred system.

Hence the objective of this study is to verify the influence of long-term storage in distilled water at 37°C (24 hours or 3 months) on the microleakage of class II resin-based composite restorations with cervical margins in dentin using 2 adhesive systems, a one-bottle and a self-etching primer system. A possible correlation between visual and morphometric methods of microleakage evaluation was also assessed.

**MATERIAL AND METHODS**

This study was approved by the Ethics in Research Committee, School of Dentistry, University of São Paulo, according to protocol number 48/02, approved in March, 2002, and report number 23/02.

Twenty sound human molars were extracted for orthodontic reasons and stored in distilled water at 37°C for a maximum post-extraction period of 90 days. Previously to cavity preparations, tooth apices were sealed using Super Bonder gel (Loctite, Itapevi, Brazil). After that, root portions were coated with 3 coats of nail polish (Nutra Nail, Up Cosmetics, Los Angeles, USA). Teeth were then

embedded in PVC tubes (Tigre, São Paulo, Brazil) using Epoxide polyester resin (Buhler, Lake Bluff, IL, USA), activated using butanox M 50 catalyst, code BUTA/100 (Redelease/c210, S.S. White, Rio de Janeiro, Brazil), maintaining the cement/enamel junction at a distance of 2 mm from the embedding resin (Figure 1).

Two class II preparations (MO/DO) were cut in each tooth with the following dimensions: 4 mm of width, 5 mm of height and 1.5 mm of depth towards the pulp. Preparations were made using number 3100 diamond burs (KG Sorensen, Barueri, Brazil) and a high-speed handpiece under constant water cooling. Cavity standardization was obtained by using a special aligning device that allowed controlled movements on the three spatial directions. Burs were replaced at each five cavity preparations.

After that, cavities were cleaned using pumice paste (S.S. White, Rio de Janeiro, Brazil) in a Robinson brush (KG Sorensen, Barueri, Brazil). MO preparations were restored using ABF experimental (Kuraray Medical Inc., Osaka, Japan) and DO were restored using Single Bond (3M ESPE, St. Paul, USA). All materials were applied strictly following the manufacturers’ instructions. Restorations were made using Z250 composite resin shade C3 (3M ESPE, St. Paul, USA) placed in increments of approximately 1 mm, so that each one was light-cured for 20 s using an Optilux 500 light-curing unit (Demetron/Kerr, Danbury, CT, USA), whose readings were maintained in 750 mW/cm².

After storage in distilled water at 37°C for 24 hours, all restorations were finished using polyester grinding papers (3M ESPE, St. Paul, USA).

For group 1 (n = 10), teeth were immediately submitted to thermal cycling in 700 cycles between water baths held at 5° and 55°C, 1 min dwell time and 3 s of interval between each bath. For group 2, teeth were again stored in distilled water at 37°C for a period of 3 months. After that, thermal cycling was carried out as described for group 1.

Upon completion, tooth surfaces were coated with 3 coats of nail polish, except for the restorative and a 1 mm rim of tooth structure. Specimens were immersed in 0.5% methylene blue solution (Fórmula & Ação, São Paulo, Brazil) for 4 hours (neutral pH). Teeth were thoroughly washed under tap water and sectioned longitudinally using a diamond disc mounted on a Labcut 1010 machine (Extec Corp., Enfield, USA) under water cooling, so that 3 sections with approximately 1 mm of thickness were obtained for each restoration. Sections were embedded in PVC tubes with 0.5 mm of height using chemically-activated acrylic resin (JET Clássico, Artigos Odontológicos Clássico, São Paulo, Brazil) in a 1:1 ratio, to protect and facilitate manipulation during microleakage assessments.

Visual analysis was performed by 3 previously calibrated examiners under a stereomicroscope with 25 X magnification (Bausch & Lomb, Rochester, NY, USA) according to the microleakage scores demonstrated in Figure 2. After scores were attributed, results were analyzed regarding examiner agreement.

The section that presented the highest microleakage score determined the final result, and was also submitted to morphometric analysis. For this study, the ImageLab 2000 image segmentation program was employed to assess dye penetration. This system consisted of a Digital Sony camera (Hyper HAD, Sony Corporation,Tokio, Japan).

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**FIGURE 1** - Schematic representation of MO and DO cavities prepared in an embedded tooth.

**FIGURE 2** - Microleakage scores employed for visual analysis of dye penetration: 0 - no dye penetration; 1 - dye penetration up to 1/3 of the gingival wall; 2 - dye penetration up to 2/3 of the gingival wall; 3 - dye penetration along the gingival wall and 4 - dye penetration reaching up to the axial wall.
adapted to a Citoval 2 stereomicroscope (Carl Zeiss Jena, Germany), which was connected to a Pavilon microcomputer (HP, Palo Alto, California, USA) equipped with a digitizer plate and the processing software ImageLab 2000 (Cambridge, Wollandport, Ontario, Canada). After image scanning, linear measurements in mm could be obtained and recorded for each section.

Firstly, to verify if there was a correlation between methods of evaluation, in which parametric and non-parametric data should be obtained, scores were turned into millimeters proportionally to the cavity size. For example: a score 1 attributed to a cavity whose axial wall was 1.8 mm deep was turned into 0.6 mm (one third of 1.8 mm); scores 0 were maintained as 0 mm, despite the cavity size. Results were submitted to Pearson’s regression analysis. Results regarding adhesive systems and storage periods were also submitted to ANOVA and Tukey’s test.

RESULTS

Regarding the two methods of evaluation, Pearson’s regression analysis presented highly significant correlation (r = 0.978, p < 0.001) between visual and morphometric methods (Graph 1), thus emphasizing that both can be reliably used and produce equivalent results.

ANOVA demonstrated no statistically significant difference between the two studied methods (p > 0.05). Regarding adhesive systems, the analysis demonstrated statistically significant differences (p < 0.001), indicating that the ABF experimental presented less microleakage when compared to the Single Bond adhesive system, either after the immediate (24 hours) or the 3-month storage period in distilled water (Table 1).

The storage period did not significantly influence the results of microleakage (p > 0.05), showing that the storage period in distilled water for 3 months had no influence on the performance of the two tested adhesive systems (Table 2).

DISCUSSION

Pearson’s regression analysis demonstrated a highly significant correlation between the tested methods of evaluation. Moreover, it is possible to observe that the reliability interval at the level of 5% varied from 0.0023 to 0.1489, showing that values obtained using both methods were very coherent. B angular coefficient (5%) went from 0.9339 to 1.0745, and hence included value 1. This coefficient indicates that a certain increase in the morphometric value corresponds to the same value obtained using score data, when we consider the cavity size.

Discrepancies were relatively higher when microleakage values were very low to be visually detected and generally corresponded to score 0 (no dye penetration). In some of these cases, the morphometric method detected a small dye penetration, which led to these discrepancies. Nevertheless, statistical analysis proved that there is a highly significant correlation between the two methods. Therefore, when equipment is available, the morphometric analysis can be used with great reliability, although the visual method revealed to be practical and efficient to evaluate microleakage, also showing that the subjectivity attributed to it does not influence the results.

TABLE 1 - Mean values of microleakage (in mm) obtained for each experimental condition.

<table>
<thead>
<tr>
<th>Method</th>
<th>Significance</th>
<th>Morphometric</th>
<th>Visual (in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhesive system</td>
<td>n.s.</td>
<td>1.04</td>
<td>1.12</td>
</tr>
<tr>
<td>ABF experimental</td>
<td>0.1%</td>
<td>0.41</td>
<td>1.75</td>
</tr>
<tr>
<td>Storage period</td>
<td>n.s.</td>
<td>24 hours</td>
<td>3 months</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>1.06</td>
<td>1.10</td>
</tr>
</tbody>
</table>

n.s.: not significant.

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Regarding adhesive systems, the ABF experimental (Table 1) showed a superior sealing ability than did Single Bond. Probably, bonding penetration occurred at the same depth as the demineralization obtained using acidic monomers\textsuperscript{11,25,26}, so that the risk of incomplete impregnation with the bonding resin does not exist, thus guaranteeing the marginal sealing observed in this study. As in previous studies\textsuperscript{11}, Single Bond presented higher microleakage when compared to the self-etching primer systems, possibly as a result of incomplete monomer penetration in the demineralized dentin zone obtained following phosphoric acid etching\textsuperscript{3,11,12,27}.

The greater microleakage obtained using Single Bond can also be explained by the fact that its bonding mechanism is based on the total removal of the smear layer following etching with phosphoric acid, which leaves an exposed collagen network available for primer impregnation. Primer application must be made after slight drying, leaving dentin sufficiently moist to prevent collapse of this collagen network\textsuperscript{22,25,27}. Due to the difficulties in verifying the ideal moisture to be left in dentin\textsuperscript{17,22,27}, marginal integrity could be compromised, resulting in higher levels of microleakage.

Some studies have already reported that bond strength decreases following long periods of storage in water\textsuperscript{2,7,16}, and that the collagen layer presents higher porosity when analyzed using scanning electron microscopy, even after 6 months\textsuperscript{21}. Considering this possible degradation, it is important to evaluate the performance of restorative materials after different storage periods. As in other studies\textsuperscript{11,16}, the period evaluated in the present study (3 months) was not enough to allow bonding degradation and consequently higher microleakage (Table 2). Li et al.\textsuperscript{11} (2001) found differences on microleakage results only after a storage period of 6 months. Hence, it is evident that more studies are necessary with longer storage periods to evaluate the longevity of these materials.

**CONCLUSIONS**

Based on the results obtained in this study, the following conclusions can be drawn:

1. The visual method of evaluation using calibrated examiners and data transformation allowed us to obtain the same conclusions as with the morphometric method, which is technologically more sophisticated.
2. The ABF experimental self-etching primer adhesive system presented significantly lower values of microleakage than did the Single Bond one-bottle system, either in the immediate assessment (24 hours) or after 3 months of storage in water.
3. The storage period of 3 months produced microleakage results similar to those produced after the 24 hour period, thus pointing out that the former is not enough to allow degradation of the bonding interface.

**TABLE 2** - Mean values of microleakage (in mm) corresponding to the method versus adhesive system versus storage period interaction.

<table>
<thead>
<tr>
<th>Method</th>
<th>Adhesive system</th>
<th>Storage period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphometric</td>
<td>ABF experimental</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>Single Bond</td>
<td>1.76</td>
</tr>
<tr>
<td>Visual (in mm)</td>
<td>ABF experimental</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>Single Bond</td>
<td>1.88</td>
</tr>
</tbody>
</table>

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