The efficacy of adhesive systems on the enamel surface has been proven; the same is not observed on dentin and cementum surfaces (Arias et al 2004). The bonding agents require etching of enamel and decalcifying of dentin to promote micromechanical bonding; for several years adhesive systems has been used phosphoric acid or similar to treat enamel and dentin substrates; those total-etching techniques remove the smear layer, open dentinal tubules and increase dentinal perme-

Microleakage of current dentin bonding systems
Microinfiltrazione marginale negli attuali sistemi adesivi

ABSTRACT

Aims: the purpose of this study was to investigate the microleakage in class V cavities restored with dental composite resin using five different dentin bonding systems (DBS): two self-etching two-components mixing, two self-etching one-component no-mixing and one total-etching.

Materials and methods: two class V cavities were prepared in 50 extracted human teeth (on buccal and on lingual surfaces) with the incisal margin in enamel and the apical margin in cementum/dentin; all the cavities were prepared using a diamond bur in a high-speed turbine handpiece with air-water spray; the teeth were randomly divided into five groups, one for each DBS, and restored using a microhybrid composite resin. Restored specimens were placed in 2% methylene blu solution for 24 h and then longitudinally sectioned for digital measurement of dye penetration.

Results: this study indicated that there was no leakage at the incisal margins (in enamel) of all the cavities, for all the DBS tested; however, none of the DBS eliminated microleakage in restorations of cavities with margins located in dentin or cementum. The results of the statistical analysis indicated the presence of significant differences among the microleakage values of the five DBS (P<0.05).

Conclusions: the restoration of cavities with margins located in dentin or cementum is an unresolved problem in operative dentistry: this study suggested that one component self-etching adhesive systems are probably more effective in reducing microleakage in dentin/cementum margins when compared with multi-steps adhesive systems.

Key words: Dentin bonding systems, microleakage, composite resin.

RIASSUNTO


Materiali e metodi: sono state allestite due cavità di classe V in 50 denti estratti con margine incisale della preparazione in smalto e apice del cementum/dentin; tutte le cavità sono state preparate con una fresa diamantata montata su turbina sotto irrigazione; i denti sono stati divisi casualmente in cinque gruppi, uno per ogni DBS, e restaurati utilizzando un composito microibrido. I campioni ottenuti sono stati immersi in una soluzione di blu di metilene al 2% per 24 ore e successivamente sezionati in senso longitudinale per la misurazione digitale del grado di infiltrazione.

Resultati: questo studio ha dimostrato che con tutti i DBS testati non si è verificata infiltrazione nei margini incisali dei restauri (in smalto); in ogni caso, nessuno dei DBS ha eliminato il rischio di infiltrazione nei restauri di cavità con margine posto in dentina o cemento. I risultati dell’analisi statistica indicano la presenza di differenze significative tra i valori di microinfiltrazione dei cinque DBS (P<0.05).

Conclusioni: il restauro delle cavità con margine in dentina o cemento costituisce un problema attualmente ancora non risolto: questo studio ha suggerito che i sistemi adesivi self-etching mono-componente sono probabilmente più efficaci nel ridurre la microinfiltrazione in dentina o cemento rispetto ai sistemi con più passaggi.

Parole chiave: Sistemi adesivi smalto-dentinali, microinfiltrazione, resina composita.

INTRODUCTION

The efficacy of adhesive systems on the enamel surface has been proven; the same is not observed on dentin and cementum surfaces (Arias et al 2004). The bonding agents require etching of enamel and decalcifying of dentin to promote micromechanical bonding; for several years adhesive systems has been used phosphoric acid or similar to treat enamel and dentin substrates; those total-etching techniques remove the smear layer, open dentinal tubules and increase dentinal perme-
ability (Fusayama et al 1979). After treatment with etchant, a primer containing one or more hydrophilic resin monomers is applied; the hydrophilic groups have affinity for dentinal surface and hydrophobic groups have affinity for resin, thereby forming the hybrid layer (Nakabayashi et al 1982); this is the bonding mechanism of most current adhesive systems. In attempt to simplify clinical procedures new dentin bonding systems (DBS) were developed consisting of a lower number of passages; DBS are currently available as three-step, two-step and single-step systems (Ferrari et al., 1997; Van Meerbeck et al., 2001; Tay et al., 2001): three-step systems are traditional adhesives, which involve the etching, the priming and the bonding steps (also defined total-etching systems); two-step systems include self-priming adhesives (that require a separate etching step) or self-etching primers (that require a separate bonding step); finally, the recently introduced single-step (self-etching) adhesives combine all bonding procedures in a single application, consisting of a mixture of acid monomers that etch enamel and dentin as well as primers that allow penetration of resin into the demineralised dentin (Nikaido, 1997). No dentin bonding agent currently available completely eliminates the microleakage at cementum/dentin interface (Prati et al., 1990; Shigetani et al., 2002; Corona et al., 2003; Abo et al., 2004; Ateyah et al., 2004; Kolinontou-Koumpa et al., 2004; Nasrien et al., 2004). The causes of microleakage are usually associated with polymerisation, shrinkage, the composite resin used, occlusal load, location of the prepared margins and the technique used (Arias et al., 2004): DBS has aim to minimize those effects. The purpose of this in vitro study was to investigate microleakage in class V cavities restored with composite resin using five different dentin bonding systems: two self-etching two-components mixing, two self-etching one-component no-mixing, and one total-etching. The null hypothesis of the study was that there is no significant difference in microleakage values among the five different adhesive systems used.

**MATERIALS AND METHODS**

**Preparation of specimens**

50 non carious vital human teeth freshly extracted for orthodontic or periodontic reasons were cleaned with scalers, polished with pumice and stored in a 0,25% mixture of sodium azide in Ringer solution until the date of use. In each tooth two class V cavities (on buccal and on lingual surfaces) were prepared by a round-nosed no. 245 carbide bur (Dentsply International, York PA, USA) at high-speed with air/water spray, according to procedure described in Corona et al. (2003): the cavities were prepared with the incisal margin located in enamel and the apical margin located in cementum/dentin (1 mm beyond the cementum-enamel junction); the dimensions of class V cavities were similar, with mesiodistal width, incisal-apical measure and depth of 3 mm. The prepared teeth were randomly assigned to five experimental groups (of 10 specimens and 20 cavities each) corresponding to the different DBS (Table 1): two self-etching adhesive systems two-components mixing (AdheSE and Clearfil Protect Bond), two self-etching adhesive system one-component no-mixing (iBond and GC Bond) and one total-etching adhesive system (Scotchbond 1 XT). After application of DBS, according to manufacturers’ directions, cavities were restored using a microhybrid composite resin (Ceram-X-Mono, Dentsply DeTrey, GmbH Germany) in three increments light-cured for 40s (Elipar Trilight-2 step mode-3M ESPE, St. Paul, MN, USA). After 24h, the restorations were finished and polished with Sof-Lex Pop-On discs system (3M ESPE, St. Paul, MN, USA) in decreasing granulation. All teeth were coated with two layers of nail varnish up to 1 mm from the restorations margins, while the apical part was sealed with wax. They were then subjected to 500 thermal cycles between 5°C and 60°C with at dwell time of 60s in each water bath. After thermocycling, the specimens were immersed in a 2% methylene blue solution and incubated at 37°C for 24 hours.

**Microleakage analysis**

The teeth were sectioned longitudinally with a low-speed water-cooled diamond cutter, viewed under a 2,5 magnification optical microscope and photographed. The images were transmitted to a personal computer and, after digitalisation, were analysed using “Digora 2.0” software (Orion Corporation Soredex, Helsinki, Finland) for measurement of dye penetration percentage at incisal and at apical margins of restorations (Figs 1-5).

**Statistical analysis**

All the data obtained were submitted to statistical analysis. Descriptive statistics including the mean, standard deviation, median, minimum and maximum values were calculated for each of the 5 groups. The results of microleakage evaluation were submitted to statistical analysis using a computer software “Stata 7.0” (Stata Corp., Station College, TX). A Kruskal-Wallis test and a Mann-Whitney U-test were performed. Significance was predetermined at P<0.05.

**RESULTS**

Representative photographs revealing dye penetration of sectioned specimens treated by each of DBS tested are presented in Figure 1-5. Images demonstrated that there was no leakage at incisal margins (in enamel) of all the cavities with DBS tested. Microleakage scores observed at apical margins (in cementum/dentin) are summarized in Table 2, which shows the relative distribution of values obtained in the test specimens, classified according to degree of leakage. The results of the Kruskal-Wallis test indicated the presence of significant differences among the microleakage values of the various groups (P<0.05). *Post-hoc* Mann-Whitney’s Test showed no significant difference among groups 1,2 and 5, (P>0.05) that all
Microleakage of current dentin bonding systems showed the highest dye penetration at apical margins. No significant difference were reported between groups 3 and 4 (P>0.05), that both showed lower dye penetration values than the other groups (P<0.05).

The null hypothesis of the study was rejected. The results of the present investigation showed significant differences in microleakage values among five different adhesive systems used. Today microleakage of composite resin restorations in enamel is significantly reduced or null. All the specimens showed no leakage at incisal margins (in enamel), according with findings in Literature (Ferrari et al., 1997; Hanning et al., 1999; Shigetani et al., 2002; Osorio et al., 2003; Corona et al., 2003; Kolinotou-Koumpa et al., 2004), with no differences emerging between the different adhesive systems; on the other side, the results of this study revealed that none of the DBS used eliminated microleakage in the apical margins located in dentin or cementum of any teeth, as confirmed by other studies (Haller, 2000; Amaral, 2001; Osorio et al., 2003).

The restoration of cavities with margins in dentin or cementum is an unresolved problem in operative dentistry; microleakage has clinical effects and causes failure of resin restorations (Fortin et al., 1994; Tay et al., 2001; Pashley et al., 2002; Nasrien et al., 2004; Tomoko et al., 2004). The reason

<table>
<thead>
<tr>
<th>Group</th>
<th>Product</th>
<th>Bonding System</th>
<th>Manufacturer</th>
<th>Mode of Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASE</td>
<td>AdheSE</td>
<td>Self-etching Two components</td>
<td>Ivoclar Vivadent AG, Schaan, Liechtenstein</td>
<td>Apply self-etching primer for 30s and disperse excess with air; apply bonding agent, dry with air and light cure for 10s</td>
</tr>
<tr>
<td>CPB</td>
<td>Clearfil Protect Bond</td>
<td>Self-etching Two components</td>
<td>Kuraray Dental Ink, Okayama, Japan</td>
<td>Apply self-etching primer for 20s and dry with mild air flow; apply bonding agent, dry gently with air and light cure for 10s</td>
</tr>
<tr>
<td>IB</td>
<td>iBond</td>
<td>Self-etching One component</td>
<td>Heraeus Kulzer, Dormagen, Germany</td>
<td>Apply self-etching adhesive system for 20s, dry with air for 5-10s to evaporate solvents and light cure for 20s</td>
</tr>
<tr>
<td>GB</td>
<td>G-Bond</td>
<td>Self-etching One component</td>
<td>GC Corporation, Tokyo, Japan</td>
<td>Apply self-etching adhesive system, leave undisturbed for 10s, gently dry with air spray and light cure for 10s</td>
</tr>
<tr>
<td>SXT</td>
<td>Scotchbond 1 XT</td>
<td>Total-etching One component</td>
<td>3M ESPE, St. Paul, MN, USA</td>
<td>Etch 20s with 37% orthophosphoric acid, rinse with water, apply DBS, gently dry and light cure for 20s</td>
</tr>
</tbody>
</table>

Table 1. Dentin bonding systems investigated. 
Sistemi adesivi analizzati.

DISCUSSION AND CONCLUSIONS

The null hypothesis of the study was rejected. The results of the present investigation showed significant differences in microleakage values among five different adhesive systems used. Today microleakage of composite resin restorations in enamel is significantly reduced or null. All the specimens showed no leakage at incisal margins (in enamel), according with findings in Literature (Ferrari et al., 1997; Hanning et al., 1999; Shigetani et al., 2002; Osorio et al., 2003; Corona et al., 2003; Kolinotou-Koumpa et al., 2004), with no differences emerging between the different adhesive systems; on the other side, the results of this study revealed that none of the DBS used eliminated microleakage in the apical margins located in dentin or cementum of any teeth, as confirmed by other studies (Haller, 2000; Amaral, 2001; Osorio et al., 2003).

Fig. 1 - Dye penetration of 2% methylene blue in sectioned specimen restored with AdheSE. 
Penetrazione del colorante (blu di metilene al 2%) nella sezione di un campione restaurato con AdheSE.

Fig. 2 - Dye penetration of 2% methylene blue in sectioned specimen restored with Clearfil Protect Bond. 
Penetrazione del colorante (blu di metilene al 2%) nella sezione di un campione restaurato con Clearfil Protect Bond.

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for this difference between apical and incisal leakage scores is that bonding to dentin or cementum is much more technique-sensitive and substrate-sensitive than bonding to enamel. There is no guarantee that bonding to dentin or cementum is as durable as bonding to enamel (Prati et al., 1990). At the apical margins none of the DBS tested was free from microleakage; furthermore, this study revealed significant differences in the degree of leakage between the dentin bonding systems. There are no relevant distinctions between two-components self-etching systems mixing (AdheSE and Clearfil Protect Bond) and total-etching system (Scotchbond 1 XT). Interesting results have been found in samples restored using self-etching adhesive systems with one component no-mixing: twice iBond and G-Bond revealed less microleakage scores than other DBS tested. According to Bedran de Castro et al. (2002), Cardoso et al. (2002) and Arias et al. (2004) this study suggested that one-component adhesive systems are probably more effective in reducing microleakage in dentin margins when compared with multi-steps adhesive systems; this may be explained by chemical composition of one-component self-etching systems as pointed out by Hayakawa et al. (2001), who stated that the best bonding on dentin was obtained when self-etching systems containing 10-MDP were used (like iBond and G-Bond). 10-MDP in fact causes minimal dissolution of smear plugs and limited opening of tubules, which reduces dentin permeability and facilitates penetration, impregnation, polymerization and entanglement of monomers with demineralised dentin to form a relatively thick hybrid layer (Osorio et al., 2003).

Table 2. Descriptive statistics of values (%) of microleakage values (apical margins) of the groups tested (each group consisted of 10 cavities).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Median</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.06</td>
<td>0.7961</td>
<td>0.9</td>
<td>2.15</td>
<td>3.1</td>
</tr>
<tr>
<td>2</td>
<td>2.05</td>
<td>0.7261</td>
<td>1.1</td>
<td>2.05</td>
<td>3.3</td>
</tr>
<tr>
<td>3</td>
<td>0.0536</td>
<td>0.0336</td>
<td>0.0063</td>
<td>0.05</td>
<td>0.11</td>
</tr>
<tr>
<td>4</td>
<td>0.0242</td>
<td>0.0238</td>
<td>0.005</td>
<td>0.015</td>
<td>0.08</td>
</tr>
<tr>
<td>5</td>
<td>2.07</td>
<td>0.6447</td>
<td>0.8</td>
<td>2.25</td>
<td>2.9</td>
</tr>
</tbody>
</table>
creased in the use of a separate etching, rinsing, drying, priming and bonding technique of total-etching adhesives or in the use of two components of self-etching adhesive systems which require application, drying and polymerization of two different products. The present study reveals that the use of one component self-etching DBS in class V composite restoration can achieve a null microleakage in enamel, comparable to that obtained by other conventional DBS, but it also documents that one component self-etching DBS can achieve a marginal integrity of restoration in dentin or cementum better to that obtained by two components self-etching or total-etching DBS. Even if in vitro dentin/cementum adhesion and DBS performances could be not the same as in vivo, the results of the present in vitro study suggest the usefulness of one component self-etching DBS in reducing failure of resin restorations. Although there has been progress in development of adhesive systems, further clinical and in vitro trials are required to confirm those data.

REFERENCES