Marginal integrity of pit and fissure sealants. Qualitative and quantitative evaluation of the marginal adaptation before and after in vitro thermal and mechanical stressing

STAVRIDAKIS, Minos M, et al.

Abstract
This research quantitatively evaluated the marginal adaptation of pit and fissure sealants. The occlusal surfaces of 48 intact, caries-free human molars were cleaned with an air-abrasion unit. The teeth were then randomly divided into eight groups of six teeth each according to the type of enamel conditioning, sealant material applied and curing unit used. After applying either 40% phosphoric acid gel (K-etch, Kuraray Co) or a self-etching primer adhesive system (Clearfil SE Bond, Kuraray Co), sealant materials of two viscosities were applied (Teethmate F-1 and Protect-Liner-F, Kuraray Co) and cured with halogen (Optilux 500, Demetron) or plasma arc (Apollo-95E, Dental & Medical Diagnostic Systems, Ltd) curing units. The marginal adaptation of the pit and fissure sealant restorations was evaluated by using a computer-assisted quantitative margin analysis in a scanning electron microscope (SEM) on epoxy replicas before and after thermal and mechanical stressing of the teeth. The results were statistically analyzed with one-way analysis of variance (ANOVA) at a confidence level of 95% (p=0.05). A post-hoc Tukey HSD-test was [...]

Reference


PMID: 12877426
Marginal Integrity of Pit and Fissure Sealants. Qualitative and Quantitative Evaluation of the Marginal Adaptation Before and After In Vitro Thermal and Mechanical Stressing

MM Stavridakis • V Favez • EA Campos • I Krejci

Clinical Relevance
The self-etching adhesive system used in this study proved to be as effective as phosphoric acid etching in the pretreatment of air-abraded enamel surface prior to sealant application. The high viscosity material performed equally well only when used in combination with the self-etching primer adhesive system as an intermediate layer. The halogen-curing unit led to better marginal adaptation than the plasma arc-curing unit, especially after thermal and mechanical stressing.

SUMMARY
This research quantitatively evaluated the marginal adaptation of pit and fissure sealants. The occlusal surfaces of 48 intact, caries-free human molars were cleaned with an air-abrasion unit. The teeth were then randomly divided into eight groups of six teeth each according to the type of enamel conditioning, sealant material applied and curing unit used. After applying either 40% phosphoric acid gel (K-etch, Kuraray Co) or a self-etching primer adhesive system (Clearfil SE Bond, Kuraray Co), sealant materials of two viscosities were applied (Teethmate F-1 and Protect-Liner-F, Kuraray Co) and cured with halogen (Optilux 500, Demetron) or plasma arc (Apollo-95E, Dental & Medical Diagnostic Systems, Ltd) curing units. The marginal adaptation of the pit and fissure sealant restorations was evaluated by using a computer-assisted quantitative margin analysis in a scanning electron microscope (SEM) on epoxy replicas before and after thermal and mechanical stressing of the teeth. The results were statistically analyzed with one-way analysis of variance (ANOVA) at a confidence level of 95% (p=0.05). A post-hoc Tukey HSD-test was used for multiple pairwise comparisons between groups. The null hypothesis was that there was no statistically significant difference between the groups that were tested in this study.
The statistically significant differences between groups were more pronounced after loading. In most cases, the self-etching adhesive system (SE Bond) proved as effective as phosphoric acid etching (K-etch). The low viscosity sealant material (Teethmate F-1), in most cases, exhibited better marginal adaptation than the high viscosity material (Protect-Liner F). The high viscosity material performed equally well only when used in combination with the self-etching primer adhesive system as an intermediate layer. The halogen curing unit (Optilux 500) led to better marginal adaptation than the plasma arc curing unit (Apollo 35E), especially after thermal and mechanical stressing.

INTRODUCTION

Dentistry's primary objective today is one of preventing dental disease rather than curing it (Tandon, Kumari, & Udupa, 1989). Pit and fissure sealants were introduced almost 35 years ago as an individual preventive method for controlling caries (Cueto & Buonocore, 1967), and their success is based on the adhesion between the sealant material and enamel due to the mechanical interlock created by the acid-etch technique. Buonocore (1955) developed this technique, which consisted of etching the enamel surface with orthophosphoric acid. Etching enamel removes surface contaminants and increases surface energy, making it easily wettable and creates an irregular surface topography of micropores and microprojections (Lee, 1969). Resin then penetrates and polymerizes in the enamel micropores, thus forming a mechanical bond with the tooth (Buonocore, 1955). Cueto (Cueto & Buonocore, 1965) presented the first report of a clinical trial using an occlusal sealing technique. Thereafter, several researchers have investigated the effectiveness of pit and fissure sealants in preventing occlusal caries in observation periods ranging from one to 15 years after application (Mertz-Fairhurst & others, 1991; Mertz-Fairhurst & others, 1995).

Conditioning enamel with phosphoric acid is the standard method for preparing the enamel surface prior to bonding sealant materials (Buonocore, Matsui & Gwinnett, 1968; Buonocore, 1955). The goals of enamel conditioning are to clean enamel, remove the enamel smear layer (when rotary instrumentation is being used), increase microscopic roughness by removing prismatic and interprismatic mineral crystals and increase the surface energy of enamel in order to produce enough monomer infiltration to guarantee the retention of resin material (Busscher, Retief & Arends, 1987; Retief, 1975). During the last five decades, many materials and different approaches have been investigated as alternatives to phosphoric acid, which was first introduced as a conditioner for hard dental tissues in a quest to achieve perfect bonding to both enamel and dentin. In order to simplify the application of resin bonding systems to both enamel and dentin, the conditioning and priming steps were combined and self-etching primers developed. They are mainly dentin conditioners combined with a hydrophilic primer (Hasegawa & others, 1989; Chigira & others, 1989). On instrumented enamel, these self-etching primers proved to be an effective alternative to conventional phosphoric acid etchants in conditioning the enamel surface in order to secure a durable bonding and marginal seal of composite resin restorations (Yoshiyama & others, 1998; Perdigao & others, 1997).

The first objective of this research was to investigate the efficacy of a self-etching adhesive system in preparing the air-abraded enamel surface prior to sealant material application. Even though most of the research with self-etching primers has been targeted on dentin adhesion, there are many indications that they can provide an effective alternative to conventional phosphoric acid etchants in conditioning the enamel surface and securing a durable bonding and marginal seal of resin restorations (Inagaki & others, 1989; Hasegawa & others, 1989). These simplified systems, by decreasing the time and steps required for placement, are convenient to use, but their efficacy has not been investigated in pit and fissure sealants.

The second objective of this research was to investigate the effect of the viscosity of the sealant material on its ability to perfectly seal pits and fissures, since contradictory reports exist in the literature. Filled sealants are preferred by some clinicians as they are more wear-resistant than unfilled sealants (Strang & others, 1986), even though a three-year clinical study showed that an unfilled light-cured resin was significantly better retained than a filled light-cured resin (Brockmann, Scott & Eick, 1989). Viscosity and flow characteristics have been reported to have no effect on the sealing ability of sealants (Low, Lee & von Fraunhofer, 1978; Barnes & others, 2000). On the other hand, Inatoda and others (2000) reported that a low viscosity sealant penetrated fully into etched enamel, whereas, the high viscosity sealants did not penetrate enough to ensure that the acid-etched enamel was sufficiently infiltrated by the sealant.

The third objective of this research was to compare the efficacy of conventional halogen curing units and plasma arc curing units when used to cure pit and fissure sealants. Within the last few years, several new polymerization concepts ("softstart" polymerization, two-step and ramped/exponential polymerization modes) and curing units (high intensity halogen curing units, plasma arc curing units, blue LED curing units and argon lasers) were introduced to the dental profession. Plasma arc curing units with high intensities and
short exposure times (one-to-three seconds), if proved efficient, would help to reduce chairtime. This would be very useful in pediatric patients, especially when multiple pit and fissure sealant applications need to be performed in one appointment, as often occurs in school-based dental public health preventive programs.

This research quantitatively evaluated the marginal adaptation of pit and fissure sealants. Human enamel cleaned by air abrasion was conditioned with phosphoric acid or a self-etching primer adhesive system and sealant materials of two viscosities were applied, then cured with halogen or plasma arc curing units. The null hypothesis was that there was no statistically significant difference between the groups tested in this study.

**METHODS AND MATERIALS**

Forty-eight intact, caries-free human molars with completed root formation were used in this study. The teeth had been stored in 0.1% thymol solution for the time between extraction and use in this in vitro test. The absence of caries was determined according to clinical parameters, using visual inspection. Only teeth without white or brown lesions that, in addition, did not exhibit cavitations, lesions including microcavities and cavities exposing dentin, were selected. After scaling and pumicing the root surface, the apices of the teeth were sealed with two coats of nail varnish and mounted on custom made specimen holders, with their roots in the center, using a cold-polymerizing resin (Paladur, Kulzer & Co, Wehrheim, Germany). The occlusal surface of all teeth was then cleaned with the help of an air-abrasion unit (AirFlow prep K1, Electro Medical Systems SA, Nyon, Switzerland) that used a high-speed stream of 25 microns aluminum oxide particles that were propelled at 4.6 bars pressure. The aim was only to clean the occlusal surface of contaminant without removing a significant amount of enamel as in the case when air abrasion is used for microcavity preparation. The use of air abrasion was chosen to clean the occlusal surface since pumice slurry and prophylactic pastes, which are often used to clean the occlusal surfaces prior to acid etching, do not completely and consistently remove debris from pits and fissures. It was of upmost importance that all debris be removed not only from the cuspal inclines (as often is the case when pumice slurry and prophylactic pastes are used), but from the complete depth of the occlusal pit and fissures, as the internal adaptation of the pit and fissure sealants would also be investigated in a subsequent part of this research.

**Table 1: Ingredients, Lot Numbers and Expiration Dates of the Materials Used**

<table>
<thead>
<tr>
<th>Material</th>
<th>Ingredients</th>
<th>LOT #</th>
<th>Expiration Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-etch</td>
<td>Phosphoric acid (40%)</td>
<td>213</td>
<td>2000-11</td>
</tr>
<tr>
<td></td>
<td>Colloidal silica Water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clearfill SE Bond*</td>
<td>MDP</td>
<td>41120</td>
<td>2000-09</td>
</tr>
<tr>
<td>Primer</td>
<td>HEMA</td>
<td>00112B</td>
<td>2001-10</td>
</tr>
<tr>
<td></td>
<td>Hydrophilic dimethacrylate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CQ</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N, N-Diethanol p-toluidine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bond</td>
<td>MOP</td>
<td>00049A</td>
<td>2001-10</td>
</tr>
<tr>
<td></td>
<td>Bis-GMA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HEMA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hydrophilic dimethacrylate</td>
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<td></td>
<td>CQ</td>
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<tr>
<td></td>
<td>N, Diethanol p-toluidine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Silanized colloidal silica</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Teethmate F-1</td>
<td>Hydrophilic dimethacrylate</td>
<td>00809</td>
<td>2000-11</td>
</tr>
<tr>
<td></td>
<td>TEG-DMA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MDP</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HEMA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CQ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protect-Liner F</td>
<td>Silanized colloidal silica</td>
<td>0040C</td>
<td>2001-02</td>
</tr>
<tr>
<td></td>
<td>Prepolymerized organic filler</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>containing colloidal silica</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bis-GMA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TEG-DMA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Methacryloy fluoride-methyl</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>methacrylate copolymer</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CQ</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Also marketed as Clearfill Mega Bond in Japan.*

**Table 2: Group Parameters Used in the Study of Marginal Adaptation of Pit and Fissure Sealants**

<table>
<thead>
<tr>
<th>Group</th>
<th>Conditioning</th>
<th>Material</th>
<th>Polymerization</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (PLH)</td>
<td>P: K-etch</td>
<td>L: Teethmate F1</td>
<td>H: Optilux 500</td>
</tr>
<tr>
<td>2 (SLH)</td>
<td>S: SE Bond</td>
<td>L: Teethmate F1</td>
<td>H: Optilux 500</td>
</tr>
<tr>
<td>3 (PHH)</td>
<td>P: K-etch</td>
<td>H: Protect-Liner F</td>
<td>H: Optilux 500</td>
</tr>
<tr>
<td>4 (SHH)</td>
<td>S: SE Bond</td>
<td>H: Protect-Liner F</td>
<td>H: Optilux 500</td>
</tr>
<tr>
<td>5 (PLP)</td>
<td>P: K-etch</td>
<td>L: Teethmate F1</td>
<td>P: Apollo 95E</td>
</tr>
<tr>
<td>6 (SLP)</td>
<td>S: SE Bond</td>
<td>L: Teethmate F1</td>
<td>P: Apollo 95E</td>
</tr>
<tr>
<td>7 (PHP)</td>
<td>P: K-etch</td>
<td>H: Protect-Liner F</td>
<td>P: Apollo 95E</td>
</tr>
<tr>
<td>8 (SHP)</td>
<td>S: SE Bond</td>
<td>H: Protect-Liner F</td>
<td>P: Apollo 95E</td>
</tr>
</tbody>
</table>
project. The occlusal surfaces were then examined under a stereomicroscope (Leica M26, Leica Microsystems AG, Wetzlar, Germany) at 4x magnification in order to check that no part of the occlusal surface was left without being air abraded and that all pits and fissures were clean of visible debris. The teeth were then randomly divided into eight groups of six teeth each based on the type of enamel conditioning, sealant material applied and curing unit used. The ingredients, lot numbers and expiration dates of the materials used in this study are reported in Table 1. The group parameters of the marginal adaptation of pit and fissure sealants used in the study are shown in Table 2.

Group 1 (PLH): The first letter (P) in the group's acronym stands for Phosphoric acid and identified the treatment of enamel prior to sealant material application. A 40% phosphoric acid gel (K-etch, Kuraray Co, Osaka, Japan) was applied with a disposable microbrush for 40 seconds in order to condition the enamel surface. The teeth were rinsed with water and air spray, using a dental syringe and thoroughly dried with oil-free compressed air. The second letter (L) in the group's acronym stands for Low viscosity, which identifies the viscosity of the sealant material. A low viscosity sealant material (Teethmate F-1, Kuraray Co) was applied with the manufacturer's disposable applicator nozzle, and the tip of an explorer was used to ensure that all pits and fissures were properly sealed. The sealant material was left intact for 20 seconds prior to polymerization to allow for a proper capillary action of resin infiltration. The third letter (H) in the group's acronym stands for Halogen curing unit, which identifies the type of curing unit used. The sealant material was light cured for 20 seconds with a halogen curing unit (Optilux 500, Demetron Research Corp, Danbury, CT, USA). The 11-mm curing tip was used to cover the entire occlusal surface in the majority of the teeth. Occasionally, when the occlusal surface of the tooth was too large, polymerization was performed for 20 seconds twice, once on the mesial and once on the distal side of the occlusal surface.

Group 2 (SLH): The first letter (S) in the group's acronym stands for Self-etching primer and identifies the treatment of enamel prior to applying the sealant material. A self-etching primer adhesive system (Clearfil SE Bond, Kuraray Co) was used to prepare the enamel surface. Clearfil SE Bond primer was applied to the entire occlusal surface with a disposable microbrush and left in place for 40 seconds before the volatile ingredients were evaporated with a mild oil-free air stream. Then, the Clearfil SE Bond adhesive resin (bond) was applied into the occlusal pit and fissures, also with a disposable microbrush, and left to penetrate for 10 seconds. With the help of a gentle oil-free air stream, the bond film was thinned before it was light-cured for 10 seconds (Optilux 500, Demetron). The same low viscosity sealant material (Teethmate F-1, Kuraray Co) was applied, and polymerization with the halogen-curing unit was performed, as previously described.

Group 3 (PHH): The 40% phosphoric acid gel (K-etch, Kuraray Co) was used to treat the enamel prior to applying the sealant material, as described in Group 1. The second letter (H) in the group's acronym stands for High viscosity, which identifies the viscosity of the sealant material. A high viscosity sealant material (Protect-Liner F, Kuraray Co) was applied with the same disposable applicator nozzle that was provided by the manufacturer of the low viscosity sealant material. The tip of an explorer was also used to ensure that all pits and fissures were properly sealed. The sealant material was left to penetrate for 20 seconds, then it was light cured in the same manner as the two previous groups.

Group 4 (SHH): The self-etching primer adhesive system (Clearfil SE Bond) was used to treat the enamel prior to applying the sealant material, as described in Group 2. The same high viscosity sealant material (Protect-Liner F, Kuraray Co) was applied as in Group 3, and polymerization with the halogen curing unit was performed in the same way as the last three groups.

Group 5 (PLP): The 40% phosphoric acid gel (K-etch, Kuraray Co) was used to treat the enamel prior to applying the sealant material, and the low viscosity sealant material (Teethmate F-1, Kuraray Co) was applied in the same way as Group 1. The sealant material was left to penetrate for 20 seconds prior to polymerization. The third letter (P) in the group's acronym stands for Plasma arc curing unit; it identified the type of curing unit used. The sealant material was light cured for three seconds with a plasma arc curing unit (Apollo 95E, Dental & Medical Diagnostic Systems, Ltd, Deurle, Belgium). The 7.6-mm curing tip rather infrequently covered the entire occlusal surface of the teeth. In a majority of the cases, polymerization was performed for three seconds twice, once on the mesial and once on the distal side of the occlusal surface.

Group 6 (SLP): The self-etching primer adhesive system (Clearfil SE Bond) was used to treat the enamel prior to applying the sealant material, and the low viscosity sealant material (Teethmate F-1, Kuraray Co) was applied in the same way as described in Group 2. Polymerization was performed with the plasma arc-curing unit, as previously described.

Group 7 (PHP): The 40% phosphoric acid gel (K-etch, Kuraray Co) was used to treat the enamel prior to applying the sealant material, and the high viscosity sealant material (Protect-Liner F, Kuraray Co) was applied in the same way as in Group 3. Polymerization was performed with the plasma-arc curing unit in the same manner as described in the previous two groups.
Group 8 (SHP): The self-etching primer adhesive system (Clearfil SE Bond, Kuraray Co) was used to treat the enamel prior to applying the sealant material, and the high viscosity sealant material (Protect-Liner F, Kuraray Co) was applied in the same way as Group 4. Polymerization was performed with the plasma arc-curing unit, as in the last three groups.

After dark storage in 0.9% NaCl normal saline solution at 37°C for one week, the sealed teeth were simultaneously loaded with repeated thermal and mechanical stresses in a chewing machine (Krejci, Albertoni & Lutz, 1990; Krejci & others, 1990). Thermal cycling was carried out by flushing water with temperatures changing 3000x from 5°C to 50°C and vice versa, with a dwelling time of two minutes each. The mechanical stress comprised 1,200,000 load cycles and was transferred on the center of the occlusal surface with a frequency of 1.67 Hz and a maximal load of 49 N. It was applied by using a natural lingual cusp in contact with the sealant's surface. The cusps that were used were taken from extracted human third molars.

Immediately upon completing the sealing procedure (from this point on referred to as initial stage) and after the thermal and mechanical stressing procedure (from this point on referred to as terminal stage), impressions of the occlusal surfaces of the teeth were made with a polyvinylsiloxane impression material (President light body, Coltène AG, Altstätten, Switzerland). Prior to the initial impression, in order to remove the unpolymerized surface layers of the sealing material and bond of the self-etching primer adhesive system due to the oxygen inhibition of the polymerization process, the occlusal surface of the tooth was cleaned for approximately five seconds with pumice slurry and a nylon bristle brush (miniature prophly brushes, Hawe Neos Dental, Bioggio, Switzerland). Subsequently, epoxy replicas (Epofix Kit, Struers, Rodovre, Denmark) were prepared for the computer-assisted quantitative margin analysis in a scanning electron microscope (Philips XL20, Eindhoven, Netherlands) at 200x magnification (Krejci, Kuster & Lutz, 1993; Krejci & others, 1990).

The marginal micromorphology was evaluated for the following qualities: "continuous margin" and "non-continuous margin." The quality criterion "non-continuous margin" was further characterized with the criteria "marginal fissure," "enamel fracture" and "sealant fracture." The different marginal qualities were assessed in percent of the total length of margins analyzed, and the values were statistically analyzed with one-way analysis of variance (ANOVA) at a confidence level of 95% (p=0.05). A post-hoc Tukey HSD-test was used for multiple pairwise comparisons between groups.

**RESULTS**

The qualitative evaluation of the marginal adaptation of the pit and fissure sealants of this study revealed that excellent marginal adaptation was feasible before and after thermal and mechanical stressing. A representative Scanning Electron Microscopy (SEM) micrograph of "continuous margin" is presented in Figure 1, where the sealing material exhibited excellent continuity with the enamel surface. Before thermal and mechanical stressing, only "marginal fissures" were encountered as "non-continuous" margins. After stressing, when discontinuity of the marginal adaptation was noted, a mixture of "marginal fissures," "sealant fractures" and "enamel fissures" was observed. A representative SEM micrograph of "marginal fissure" is presented in Figure 2, where the gap is localized at the enamel/sealant interface. The "sealant fractures" that were encountered were, in most instances, of a small magnitude and localized at the periphery of the margins of the sealants as illustrated in Figure 3. In the high viscosity sealant material groups that were polymerized with the plasma arc curing unit (Group 7...
(PHP) and Group 8 (SHP) "sealant fractures" of greater magnitude, as illustrated in Figure 4, that extended over all the occlusal surface were encountered. "Enamel fractures" were scarcely observed, primarily around the occlusal contact points.

Table 3 summarizes the quantitative evaluation results of the marginal adaptation of the different groups of pit and fissure sealants tested in this study, where the mean percentage of "continuous margin" of each group before (initial) and after (terminal) thermal and mechanical stressing, and the statistical significance between groups are reported. Statistical analysis of the "non-continuous margin" results were not performed, as these percentages were complimentary to the percentages of "continuous margin." The results of the different types of "non-continuous margin" only at the terminal stage are reported in Table 4, since at the initial stage, only "marginal fissures" were encountered.

In most cases, the self-etching adhesive system (Clearfil SE Bond) proved to be as effective as phosphoric acid etching (K-Etch) in the pretreatment of the enamel surface prior to sealant application. Statistically significant differences in pairwise comparisons with phosphoric acid etching were observed in three situations. In two of them (initial: Group 7 (PHP) versus Group 8 (SHP) and terminal: Group 5 (PLP) versus Group 6 (SLP)), where the plasma arc curing unit was used, the phosphoric acid etching groups exhibited better marginal adaptation. In both self-etching primer adhesive system groups (initial: Group 8 (SHP) and terminal: Group 6 (SLP)), there was one sample in each group that behaved very poorly, which increased the standard deviations of these groups. In the third situation, (terminal: Group 3 (PHP) versus Group 4 (SHP)), when the halogen-curing unit was used, it was the self-etching primer adhesive system group that produced better marginal adaptation.

The low viscosity sealant material (Teethmate F1), in most cases, exhibited better marginal adaptation than when the high viscosity material was used (Protect-Liner F). The statistically significant differences in the pairwise comparisons between the low and high viscosity sealant materials were in favor of the low viscosity sealant material in all four situations where they were observed (initial: Group 6 (SLP) versus Group 8 (SHP); terminal: Group 1 (PLH) versus Group 3 (PHP), Group 5 (PLP) versus Group 7 (PHP), and Group 6 (SLP) versus Group 8 (SHP). In only one case did the high-viscosity sealant material exhibit better marginal adaptation (initial: Group 5 (PLP) versus Group 7 (PHP)), however, the difference was not statistically significant.

The halogen-curing unit (Optilux 500) generally led to better marginal adaptation than the plasma-arc curing unit (Apollo 95E). The differences were statistically significant in all except three pairwise comparisons (initial: Group 1 (PLH) versus Group 5 (PLP), Group 2 (SHP) versus Group 6 (SLP) and Group 3 (PHP) versus Group 7 (PHP)).
The statistically significant differences between groups were more pronounced in the terminal stage. Prior to stressing, there were just two subsets with statistically significant differences between them, and seven out of eight groups had percentages that exceeded 80% of excellent marginal adaptation. After stressing, there were four subsets with statistically significant differences between them. The variations in the percentage of excellent marginal adaptation were large, as the best subset exceeded 90% of “continuous margin,” while the worst exhibited less than 10% of “continuous margin.”

Three out of four groups in which the halogen curing unit was used (Group 1 (PLH)), Group 2 (SLH) and Group 4 (SHH) did not exhibit statistically significant differences between the initial and terminal situation. Thermal and mechanical stressing caused a statistically significant decrease in the marginal adaptation only in one of the halogen curing unit groups (Group 3 (PHH)).

All groups in which the plasma arc curing unit was used (Group 5 (PLP)), Group 6 (SLP), Group 7 (PHP) and Group 8 (SHP) exhibited statistically significant differences for the criterion “continuous margin” between initial and terminal situation. In all of these groups, after thermal and mechanical stressing, there was a statistically significant decrease in the percentage of “continuous margin.”

DISCUSSION

The results of this study support the use of self-etching primers as effective means for preparing air-abraded enamel for bonding of a fissure sealant. Air abrasion removes the outer enamel layer of a few microns thickness and induces an additional micro-etching pattern (Figure 5) that could enhance the efficacy of the self-etching primers. Removing this small layer of external enamel may have contributed to the efficacy of the self-etching adhesive system in conditioning the enamel surface prior to applying the sealant material. The acidic, self-etching primer was capable of creating an etching pattern on the enamel surface (Figure 6) which was less profound than when phosphoric acid was used (Figure 7). The results of this research cannot support the efficacy of the self-etching primer that was studied on non-prepared intact enamel, since the surface on which it was applied had been previously air abraded. This does not diminish the value of the results of this research project since air abrasion was performed in order to thoroughly clean the enamel surface from plaque, pellicle and other contaminants (Figure 8) and not to facilitate the action of self-etching primer, such as prophylactic pastes and pumice slurry, which are often used to clean the occlusal surfaces prior to acid etching and do not completely and consistently remove debris from pits and fissures (Figure 9).
It is also important to note that the self-etching primer was not applied alone, but the bond of the adhesive system was also applied according to the manufacturer’s recommendations. Therefore, the true comparison between phosphoric acid and self-etching primers was not actually performed, even though there are strong indications to support the results of this research that self-etching primers may perform equally well. Currently, there are ongoing investigations where the self-etching primer was solely applied (without the bond) in order to compare phosphoric acid etching versus self-etching primer conditioning. Another issue is the fact that these positive results cannot be extrapolated to other invasive techniques, such as mechanical widening of the fissures with rotary instrumentation or when lasers are applied, unless the necessary research is performed.

The clinical consequences of etching enamel with weaker acids, and the presence of dissolved calcium phosphates and/or aluminum oxide particles that cannot be removed with rinsing which are then entrapped in the bonding resin layer are issues that certainly need to be thoroughly investigated. A recent, long-term *in vivo* study of the durability of self-etching primer bonded restorations suggested that the bond failure of the aged interfaces occurred through the loss of resinous material that probably occurred through water sorption and hydrolysis of the hydrophobic resin components (Sano & others, 1999). Therefore, long-term clinical longitudinal studies, where retention rates and mar-
ginal adaptation evaluation are examined, are needed in order to further investigate the positive results of this *in vitro* study, in which the self-etching adhesive system that was used proved to be as effective as phosphoric acid in conditioning air-abraded enamel prior to applying sealant.

The low viscosity sealant material exhibited better marginal adaptation than its high viscosity counterpart. Busscher and others (1997) suggested that a time-delay be introduced between the time of application and light activation when curing light-cured sealants to allow for a proper capillary action of resin infiltration. This is not related to viscosity but rather to diffusion kinetics as driven by the 2nd Fick's law. Nevertheless, the highly filled viscous sealant material performed equally well when used in combination with the self-etching primer adhesive system as an intermediate layer. The use of an intermediate bonding agent has been proposed in order to provide better flow of thick and viscous highly filled sealants which are difficult to spread into small fissures. Simonsen (1982) proposed using an unfilled resin as an intermediate layer under a filled sealant when placing a small preventive resin restoration. The bonding agent serves as a low-viscosity, flowable wetting agent for the interface between etched enamel and filled sealant, so that the viscous sealant spreads better and properly wets the surface of the fissures (Simonsen, Chu & Meyers, 1996; Davenport & Feigl, 1997). The results of this research seem to agree with this concept, since the high viscosity sealant material, when used in combination with the adhesive system and cured with the halogen curing unit (Group 4 (SHH)), resisted the thermal and mechanical stressing in a more favorable manner than when applied in etched enamel without the intermediate bonding layer (Group 3 (PHH)). Nevertheless, studies on marginal adaptation, alone, are not enough to substantiate the claim that an intermediate bonding agent provides better flow for highly filled sealants with a higher viscosity. Internal adaptation studies currently underway are definitely required before such a claim can be supported.

The plasma arc-curing unit did not prove its efficacy in providing excellent marginal adaptation after thermal and mechanical stressing. The efficacy of plasma arc curing units has been investigated in orthodontic bonding, where saving time is of major importance since numerous brackets need to be bonded in one appointment. There are reports that support the use of plasma arc curing units as advantageous alternatives to halogen curing units for orthodontic bonding (Ishikawa & others, 2001; Sfondrini & others, 2001). Even though one- and two-seconds exposure periods of plasma arc light were found incapable to produce adequate bonding, three-second exposure periods produced results similar to 20 seconds of halogen light (Pettenerides, Ireland & Sherriff, 2001). The results of this research suggest that one three-second exposure was not sufficient to cure the sealing materials and the adhesive system that were used. Six or nine second exposures have been reported as necessary to create bond strengths equal to those produced by exposure to 40 seconds of tungsten-quartz halogen light (Oesterle, Newman & Shellhart, 2001). If several polymerization cycles were used, the results of this study might have been different. Nevertheless, even if a great number of cycles were proven to be necessary, keeping in mind that most often two polymerization cycles were needed due to the limited diameter of the curing tip, this would make the main advantage of plasma-arc curing units, which is short time curing periods, obsolete and their higher cost would not be justified.

One explanation for the poor results might be the narrow spectrum of the emitted wavelength that might have been incompatible with the materials used in this study. Most composites are activated by the camphorquinone/amine system for which maximum absorption occurs at 468 nm. Materials using photoinitiators that have absorption maxima lower than camphorquinone (468 nm) may not be properly activated by the plasma-arc curing unit used (Stansbury, 2000). Apollo 96E produces considerably more intensity over a rather narrow wavelength range around this value and is more specific to the activation of camphorquinone. However, the very short exposure period used offers low total irradiation energy to the system, which leads to incomplete conversion, especially at indith regions (Nomoto, 1997).

Another explanation for the poor results may lie in the kinetics of polymerization for the camphorquinone/amine system. It has been reported that relatively high exposure intensities and short curing times may result in compromised properties in the cured resin, as they may result in an increased early termination of polymer chains in the pre-gel polymerization period (Koliniotou-Kubia & Jacobsen, 1990; Kelsey & others, 1992). Munksgaard, Peutzfeldt and Asmussen (2000) reported that the elution of BISOMA and TEGDMA from experimental resin specimens and commercially available resin composite specimens cured with a plasma arc light unit (three seconds) was seven and four times higher, respectively, compared to elution from specimens cured with a halogen unit (40 seconds). This high amount of leachable monomers in a resinous material may indicate poor conversion of monomer to polymer and, consequently, poor mechanical properties. Plasma arc curing units have been reported to result in resin composite materials with inferior mechanical properties (surface hardness, flexural strength and modulus of elasticity [Sharkey & others, 2001; Hofmann & others, 2001]). Nevertheless, there is one report in the literature where a plasma arc curing unit sufficiently polymerized pit and fissure sealants and
produced restorations without microleakage (Stritikus & Owens, 2001). In that study, a different sealing material was applied, then cured with a different plasma arc curing unit for a longer curing period, and the sealant restorations were thermocycled for only 200 cycles, while no mechanical load was applied. It is possible that the poor results obtained in this research may be due to incompatibility between the materials used and the plasma-arc curing unit and should not be generalized to all materials and all plasma-arc curing units. As newer generations of plasma-arc curing units are introduced into the dental profession, they need to be evaluated with different pit and fissure sealants.

Due to the "coastline" periphery of the margins of pit and fissure sealants, it is nearly impossible or of little scientific value to conduct marginal adaptation evaluation with the help of microphotographs where the margin is simply counted as open or closed in certain predetermined areas, and the magnitude of the marginal discrepancy is recorded in terms of microns. In this study, the quality of the marginal adaptation of the pit and fissure sealants was evaluated with a computerized quantitative technique that was first described by Krejci, Lutz and Loher (1991) in the entire enamel-sealant external interface and not in a few locations of the periphery. Another advantage of this evaluation method is that it was a non-destructive technique. By working with epoxy replicas, evaluation of the marginal adaptation, both before and after mechanical and thermal stressing, was performed on the same pit and fissure sealant restoration.

In other destructive kinds of evaluation techniques, such as microleakage studies, often, each specimen has to be dissected in order to be evaluated. Because of that, most often, evaluation is performed only after the stressing (most often only thermocycling) procedure in order to reduce the number of specimens. In the case of poor results, it is not possible to differentiate whether the failure was caused by the stressing procedure or if the sealing procedure did not perform adequately from the beginning. With this research methodology, if the same specimens were evaluated before and after thermal and mechanical stressing, any deterioration of the marginal adaptation could only be the consequence of the stressing procedure.

Another point that needs to be emphasized is the usefulness of the stressing procedure as it helped to depict which groups performed favorably. Before stressing, the majority of the groups performed equally well, while after stressing, there were only a few groups that exhibited excellent marginal adaptation. After stressing, there were four, instead of two, subgroups that exhibited statistically significant differences between them, while the comparison between the initial and terminal stage also revealed some interesting points. Although fissure sealants are not recommended for occlusal contact (Heinrich-Weltzien & Kühnisch, 1999), in many clinical cases, this is difficult to realize. The stressing conditions applied in this in vitro study did not exceed the physiological range. The temperature of the water used for thermocycling (Palmer, Barco & Billy, 1992) and the chewing forces (Bates, Stafford & Harrison, 1975) were chosen according to physiologic data. The high number of load cycles represent a clinical service time of about five years (Krejci & Lutz, 1990). Therefore, the results of this study may be clinically more relevant in predicting long-term behavior than shear bond strength on flat enamel surfaces where no attempt is made to simulate cavity geometry or investigations where only thermal cycling or only short mechanical loading with extremely high chewing forces were applied (Krejci & others, 1994).

CONCLUSIONS

The self-etching primer adhesive system used in this study proved to be as effective as phosphoric acid in preparing air-abraded enamel for fissure sealing. The low viscosity sealant material exhibited better marginal adaptation than its high viscosity counterpart. The highly filled viscous sealant material performed equally well only when used in combination with the self-etching primer adhesive system as an intermediate layer, which probably played the role of a low-viscosity, flowable wetting agent. The halogen-curing unit resulted in better marginal adaptation than the plasma-arc curing unit, especially after thermal and mechanical stressing. Ongoing in vitro research that is currently underway will try to address some of the issues raised by this research project, while at the same time, long-term clinical studies are certainly needed in order to confirm the results of this in vitro study.

Acknowledgements

The authors express their gratitude to Marie-Claude Reymond for her most valuable technical assistance. Product support for this study was kindly provided by Kuraray Co (Osaka, Japan).

(Received 12 July 2002)

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