IN VITRO STUDY OF MICROLEAKAGE OF FISSURE SEALANT WITH DIFFERENT PREVIOUS TREATMENTS

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ABSTRACT
The purpose of this work was to evaluate microleakage of a sealant after using three different techniques for conditioning the surface to be sealed. Twenty-four caries-free upper and lower premolars were used, which were preserved in distilled water at room temperature. The structural faults were enlarged using a cylindrical conical diamond (ISO 007). Teeth were randomly assigned into three groups of eight. Group I (control) was conditioned with 37% phosphoric acid (Vivadent) for 15 seconds after which the sealant Helioseal F (Vivadent) was applied and cured for 40 seconds. Group II was conditioned in the same way, after which one-step adhesive Te-econom (Vivadent) and the sealant were applied. Group III was conditioned using a self-etching adhesive, Go (SDI), after which the sealant was applied. Adhesive was applied according to the manufacturer’s instructions. The samples were thermocycled for 300 cycles between 5º and 55ºC and immersed in a 2% methylene blue solution for 48 hs. at standardized temperature of 37°C ± 1º. Then they were rinsed with tap water and ground longitudinally in V-P direction with silica carbide rotatory disks of decreasing grit. The amount of leakage was evaluated under stereoscopic microscope at 40X magnification. The longitudinal penetration of dye into the tooth-sealant interface was scored on a scale of 0 to 3. The results were analyzed by a Kruskal-Wallis non-parametric test. In Group II, 100% of the samples showed low (50%) or no (50%) leakage. Both the other groups had a higher percentage of specimens with high leakage (scores 2 and 3) (P = 0.000). Group II had the best performance, with significant differences (P = 0.0028) compared to the other experimental groups. Marginal leakage was lowest when the tooth was conditioned with phosphoric acid and subsequent application of an adhesive.

Key words: pit and fissure sealants; dental leakage; dental etching.

ESTUDIO IN-VITRO DE LA MICROFILTRACIÓN DE UN SELLADOR DE FOSAS, SURCOS Y FISURAS CON DISTINTOS TRATAMIENTOS PREVIOS A SU APLICACIÓN

RESUMEN
El objetivo de este trabajo fue evaluar la microfiltración de un sellador con tres técnicas diferentes de acondicionamiento previo de la superficie a sellar. Se utilizaron 24 premolares superiores e inferiores, libres de caries, conservados en agua destilada a temperatura ambiente. Los defectos estructurales fueron ensanchados empleando una piedra de diamante cilíndrico (ISO 007). Los dientes fueron aleatoriamente asignados en 3 grupos de 8 elementos cada uno. Grupo I (control) fue acondicionado con ácido fosfórico al 37% (Vivadent) por 15 segundos, posteriormente se aplicó el sellador Helioseal F (Vivadent) y se fotopolimerizó durante 40 segundos. Grupo II fue acondicionado de la misma manera, con un adhesivo monoenvasado Te-Econom (Vivadent) y el sellador correspondiente. Grupo III fue acondicionado con un adhesivo autograbado, Go (SDI), y el sellador. Los adhesivos se aplicaron de acuerdo con las instrucciones del fabricante. Las muestras fueron termocicadas por 300 ciclos entre 5º y 55ºC y sumergidas en una solución de azul de metileno al 2% durante 48 hs. a una temperatura estandarizada de 37ºC ± 1º. Luego se enjuagaron abundantemente con agua corriente, se desgastaron longitudinalmente en sentido V-P sobre discos rotatorios de carburo de silicio de grano decreciente. La colocación del colorante se hizo al ensamblar la interfase diente-sellador, bajo lupa estereoscópica a 40X. La penetración longitudinal del colorante fue registrada en una escala de 0 a 3. Los resultados fueron analizados mediante la prueba no paramétrica de Kruskal-Wallis. En el Grupo II, 100% de las muestras presentaron baja (50%) o nula (50%) filtración. Los otros grupos presentaron un mayor porcentaje de muestras con filtración importante (2 y 3) (P = 0.000). Se puede determinar que el mejor comportamiento se obtuvo en el Grupo II con diferencias significativas (p = 0.0028) con respecto a los otros grupos. La menor filtración marginal se obtuvo cuando el diente fue previamente acondicionado con ácido fosfórico y posterior aplicación de un adhesivo.

Palabras clave: selladores de fosas y fisuras; filtración dental; grabado dental.
INTRODUCTION
The diagnosis of pit, groove and fissure caries is one of the major problems faced by general and pediatric dentists. The complex, irregular, unpredictable shape of those parts of the tooth favors the formation of caries, and makes diagnosis difficult, complicated and sometimes impossible by means of classical methods of exploration and diagnosis1. Current preventive strategies tend to be fluoride administration and the use of pit and fissure sealants, among others. The structural defects of the occlusal faces are areas that favor plaque retention and where the action of fluoride is less effective. In order to prevent caries from developing in these zones, pit, spot and fissure sealants have been developed successfully, and are used as an effective, minimally invasive preventive procedure2. These sealants form a physical barrier that isolates the surfaces from the oral environment, preventing accumulation of organic debris and the combination of bacteria, their nutrients and acid metabolized products, in addition to preventing the development of incipient lesions3-4.

The effectiveness of sealants has been the object of much research. Nevertheless, it is not clear which procedure provides the best results5. Some authors have studied retention, penetration capacity and application technique to determine efficacy6-8; others have attempted to demonstrate the influence of invasive methods on the penetration capacity of fissure sealants3-10. Simonsen et al.4 reported that enlarging a fissure with a drill enables better penetration and adaptation of the sealant compared to the conventional technique with untreated fissures. Enameloplasty also enables a better diagnosis of decalcification in the part nearest to the occlusal surface of the fissure, elimination of remaining debris and increase of the surface area for retaining the material11.

There are publications evaluating penetration and microleakage capacity of conventional sealants, fluid composites or composites and glass ionomers, based on the degree of viscosity7,12,13. Other studies have shown that better adaptation and retention can be achieved when an adhesive agent is applied between the enamel and the sealant14,15. These adhesives may be conventional or self-etching.

However, it has not yet been determined which the best procedures is for achieving clinical success. It should also be noted that the fluoride ions in restorative materials have preventative effects. This began with the use of conventional glass ionomers (GI) also used as sealants, in their lower viscosity presentation (Type I) due to their high fluoride release, and led to the use of fluorides in different alternative materials used as sealants. One of our studies showed that fluid composites and composites released significantly less fluorides than glass ionomers did, although they did so in a more continuous and sustained manner up to the end of the study period (60 days). In addition to the low viscosity and better marginal sealing capacity provided by the adhesive technique, this would make them a good alternative as adamantine pit and fissure sealants2.

Ideally, sealants should remain adhered to the surface of the tooth for long periods of time and should not allow microleakage under them or between them and the enamel surface5.

This study evaluated microleakage of a sealant after using three different techniques for prior conditioning of the surface to be sealed, in order to determine whether the use of new adhesives significantly reduces leakage.

MATERIALS AND METHODS
Twenty-four lower and upper caries-free premolars which had been extracted for orthodontic purposes were used. They were stored in distilled water at room temperature until they were used. The adamantine structural defects of the occlusal surface of the teeth were enlarged using an ISO 007 standard cylindrical-conical diamond bur (Braseller-Komet/Germany), to see whether the groove was caries-free and remove any organic debris from inside the fissure, to a width of approximately 1/8 of the inter-cusp distance.

Then the teeth were randomly distributed into 3 groups of eight teeth each according to the treatment of the adamantine surface before applying the sealant (Table 1). In groups I and II, the enamel was etched with 37% phosphoric acid for 15 seconds, washed for 45 seconds and dried for 30 seconds. The adhesives for groups II and III were applied according to manufacturers’ instructions. A light-curing sealant was used, which has a filler with fluoride releasing capacity Helioseal F (Vivadent). The material was cured for 40 seconds using a high-powered...
visible light emitting device, 850 mW/cm² (XL 3000-3 M/ESPE).
The test specimens were stored in distilled water at 37°C for 24 hours. Then they were thermocycled between 5°C and 55°C in water for 300 cycles, with a dwell time of 30 seconds.
Then the teeth were sealed apically with resin and the specimens entirely coated with two layers of nail varnish to within 1 mm of the tooth-sealant junction. They underwent leakage tests by immersion in a 2% aqueous methylene blue solution for 48 hours and stored in an incubator for bacterial culture at a standardized temperature of 37°C ± 1°C. After being removed from the dye and washed in plentiful water, they were ground in vestibular-palatal direction to a medial plane in a metallographic polisher (CIM, Centro de Investigación de Materiales. UNC) using decreasing grit silicon carbide rotating discs, at low speed with water refrigeration, and observed and microphotographed under stereoscopic microscope at 40 X magnification.
The amount of leakage was determined by scoring the depth to which the dye penetrated on a scale of 0 to 3 (Table 2).

RESULTS

Table 3 shows the percentage of samples that leaked for each treatment. It is noticeable that for Group II, in 100% of the samples there is little (50%) or no (50%) leakage (p=0.000). In both the other groups there is a high percentage of samples with high leakage scores (2 and 3) (p=0.000).
Leakage was scored on a scale of 0 to 3 (0 = least leakage, 3 = most leakage), and two readings were performed on each sample to obtain a single mean value.
This variable therefore lacks the metric qualities needed for analysis of variance, so the results were subject to non-parametric statistical studies using a Kruskal-Wallis test.
Microleakage was lowest in Group II, in which the adamantine surface was conditioned using 37% phosphoric acid followed by application of Te-Econom one-step adhesive. There were significant differences (p= 0.0028) with Groups I and III, both of which behaved in similarly and exhibited more microleakage.
The microphotographs show some of the determinations confirming the results reported in this study (Figs. 1 to 4).

Table 1. Previous Treatments.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Manufacturer</th>
<th>Lot Nº</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Control)</td>
<td>Conditioning with 37% phosphoric acid</td>
<td>Ivoclar Vivadent AG Schaan. Liechtenstein</td>
<td>42016</td>
</tr>
<tr>
<td>II</td>
<td>Conditioning with 37% phosphoric acid and application of one-step adhesive (Te-Econom)</td>
<td>Ivoclar Vivadent AG Schaan. Liechtenstein</td>
<td>3742</td>
</tr>
<tr>
<td>III</td>
<td>Conditioning with a self-etching adhesive (Go)</td>
<td>Southern Dental Industries (SDI) Australia</td>
<td>80306</td>
</tr>
</tbody>
</table>

Table 2: Criteria for determining amount of marginal leakage.

<table>
<thead>
<tr>
<th>Level</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No penetration of dye</td>
</tr>
<tr>
<td>1</td>
<td>Superficial penetration</td>
</tr>
<tr>
<td>2</td>
<td>Lateral penetration</td>
</tr>
<tr>
<td>3</td>
<td>Penetration under the sealant</td>
</tr>
</tbody>
</table>

Table 3: Number and percentage of samples that leaked in each experimental treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leakage level</th>
<th>Nº / % of samples that leaked</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0</td>
<td>0 / 0%</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1 / 12.5%</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4 / 50%</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3 / 37.5%</td>
</tr>
<tr>
<td>II</td>
<td>0</td>
<td>4 / 50%</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>4 / 50%</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0 / 0%</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0 / 0%</td>
</tr>
<tr>
<td>III</td>
<td>0</td>
<td>1 / 12.5%</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2 / 25%</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3 / 37.5%</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2 / 25%</td>
</tr>
</tbody>
</table>
DISCUSSION

Sealing grooves, pits and fissures is considered to be an important procedure among the strategies for preventing or reducing the risk of caries in their initial stages\(^\text{10}\). Chan et al.\(^\text{16}\) report that in order to reduce microleakage significantly, it is essential that the sealing material should fit perfectly, and this may be directly related to the mechanical preparation of surface defects and the retention and longevity of the sealant compared to untreated fissures\(^\text{9}\). Therefore, the occlusal surfaces of the teeth used in this study were enlarged using a cylindrical-conical diamond bur standard ISO 007, to a width of 1/8 of the intercusp distance. Several studies have proved that fit and retention can be improved by applying an adhesive between the enamel and the sealant\(^\text{14-15}\). Pulgar et al. also report that applying an adhesive before the sealant improves retention, lowers the susceptibility of the technique to moisture and reduces microleakage, and that it is essential when using composites with medium percentages of filling, such as fluids. Moreover, it does not take longer than conventional techniques\(^\text{2}\).

Recently introduced self-etching adhesives are composed of acidic monomer solutions. This provides simultaneous capacity to etch and condition the hard dental tissues and penetrate with acidic molecules that can cure “in situ”, eliminating the need for the prior acid etching, washing and drying clinical steps. Moreover, it eliminates concern regarding moisture control, which is usually a critical aspect of resin-enamel adhesion\(^\text{17-19}\). Using these adhesives therefore seems to be an attractive alternative to the acid etching technique for applying a sealant in children and young people, when the clinical procedure needs to be simplified. This study used sealant microleakage to evaluate the influence of three different types of prior conditioning of the surface to be sealed.

The results showed that conditioning with 37% phosphoric acid and subsequent application of one-step adhesive before applying the sealant significantly reduces microleakage compared to the conventional technique or to using self-etching adhesive. These findings match previous studies by Pulgar et al.\(^\text{12}\) and Iridona et al.\(^\text{13}\), while they disagree with the results reported by Simancas Pereira et al.\(^\text{8}\) and Peutzfeldt et al.\(^\text{17}\), among others. The structural changes produced by etching with phosphoric acid, and the moisturizing capacity and viscosity of the adhesive are essential for the resin to infiltrate the enamel, thus reducing leakage\(^\text{13}\). The acidity of self-etching adhesive systems is much lower than that of phosphoric acid, therefore they do not etch enamel as effectively, especially if the adamantine tissue has not been previously prepared or instrumented\(^\text{18}\).

Moreover, although self-conditioning adhesive systems attain acceptable adhesion values on unetched enamel, these values increase significantly when the surface is conditioned. Furthermore, self-etching adhesives would attain higher adhesion values on dentine than on adamantine tissue\(^\text{19}\).

The patterns of adamantine etching are not equivalent to those achieved with phosphoric acid, so there is less benefit for adhesive interaction. This may be due to...
poor penetration of acidic monomers in the surface of the enamel, some mineral precipitation altering the
depth of demineralization, or the difficulty of diffusing
in interprismatic spaces in the case of certain adhesives
with filler\textsuperscript{20}. Some authors believe that it would be
recommendable to acid-etch the enamel before appl-
ing a self-etching adhesive\textsuperscript{21}. Moreover, it is impor-
tant to consider that the enamel surface has an
aprismatic configuration in the zone of occlusal fissu-
res. Therefore, treatment with self-etching agents does
not eliminate a significant amount of the surface layer
of enamel without prisms, since it is not washed after
applying to the tissue. The aprismatic structure of the
enamel might prevent penetration of self-etching adhe-
sives, leaving some zones partly unetched and with
inadequately sealed fissures\textsuperscript{22-24}.

To sum up, all of the above would justify the results
obtained in this study and the greater leakage observed
in Group III, in which a self-conditioning adhesive was
applied before the sealant, and which behaved in the
same way as the control group without adhesive.

CONCLUSIONS

- The type of enamel conditioning had a significant
influence on marginal sealing capacity.
- Marginal leakage was lowest when the treatment prior
to sealant application was phosphoric acid condi-
tioning followed by the application of one-step adhesive.