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Effect of saliva contamination on microleakage of three different pit and fissure sealants

ABSTRACT

Alm The aim of the study was to evaluate the effect of saliva contamination and compare the microleakage of three different pit-and-fissure sealants namely, Helioseal F, Enamel Loc and Fuji VII.

Methods Sixty recently extracted sound third molars were randomly assigned to three groups for three different sealant materials (n=20). Each sealant group was then randomly divided into two as uncontaminated (n=10) and saliva contaminated (n=10) prior to sealant placement. The samples were thermocycled in water for 500 cycles between 5 and 55°C with a dwell time of 30 s and immersed in 1% methylene-blue for 24 h. The samples were sectioned and scored on a 3 point rating scale using a light microscope.

Results Among both contaminated and uncontaminated groups Helioseal F showed statistically significantly less microleakage compared to Enamel Loc and Fuji VII groups (p<0.05). There was no statistically significant difference between Enamel Loc and Fuji VII in both absence and presence of saliva contamination (p>0.05).

Statistics The results were analysed using Kruskal Wallis test while study groups were compared with Mann-Whitney test for statistically significant differences at 5% significance level.

Conclusion Under both uncontaminated and saliva contaminated conditions, the light-cured resin-based pit-and-fissure sealant Helioseal F yielded lower microleakage scores compared to Enamel Loc and Fuji VII.

Keywords: microleakage, fissure sealant, uncontaminated, saliva contaminated.

Introduction

Evidence suggests that nearly 90% of caries in children occurs in pit and fissures [Weintraub, 2001]. This derives from the complex anatomical characteristics of pit and fissures that hampers plaque removal and penetration of fluoride and bactericide solutions within the fissures [Carvalho et al., 1989; Fennis-le et al., 1998; Pearce et al., 1999].

Pit and fissure sealants constitute the preventive interventions in paediatric dentistry and their effectiveness for caries management on occlusal surfaces has been documented in numerous clinical studies [Locker et al., 2003]. Since the inception of fissures sealants, a number of types of sealants have been developed varying according to the base material, the method of polymerisation and fluoride release ability [Duke, 2001]. It is well known that, marginal sealing ability of fissure sealants is prerequisite for successful treatment. In cases of lackness of an effective sealing, microleakage occurs resulting in possible caries lesion progression underneath the sealant material [Kidd, 1976]. It is noteworthy to underline that, following the correct indication for sealing, isolation of the tooth is the most important aspect of sealant placement since sealing of pit and fissures always brings the risk of contamination which influences an effective seal [Locker et al., 2003]. Unfortunately, complete isolation of the tooth from contamination by saliva is very difficult considering the multi-steps involved in the placement of pit and fissure sealants particularly in uncooperative children [Ashwin, 2007].

New alternatives are in market today in an attempt to achieve better results by simplifying the application steps. Among them, light-cured self-etching resins and glass-ionomer cements eliminate acid etch, rinse and dry steps which might require less chair-time.

The aim of this in vitro study was to evaluate the effect of saliva contamination and compare the microleakage of three different pit-and-fissure sealants namely, light-cured unfilled resin based; Helioseal F (Ivoclar, Vivadent AG, Germany), light-cured self-etching resin-based; Enamel Loc (Enamel Loc, Rev, USA) and glass-ionomer cement Fuji VII (GC Corporation, Tokyo, Japan).

Materials and methods

Sixty recently extracted sound impacted third molars were kept in distilled water in a room temperature of 23°C to be used in the study. The fissures were cleaned with pumice using a soft brush and air-water jet. There were no cracks analysed on the surface of the teeth which were randomly divided into three groups for three different sealant materials to be placed. Details of the pit and fissure sealant materials are given in Table 1. Each sealant group was then randomly divided into two as uncontaminated (n=10) and saliva contaminated (n=10) prior to sealant placement. Saliva contamination was done by applying 0.1 ml fresh human saliva supplied from one person on the surface of pit and fissures of the teeth and leaving undisturbed for 10 s. Only in Helioseal F group saliva contamination was done after etching of the enamel. Following contamination, the surface was air dried prior to sealant application for all three groups.

Sealant treatment

The sealant materials were placed according to the manufacturer’s instructions as described below. In Helioseal F group, for etching, 37% phosphoric acid was applied on pit and fissures for 30 s, rinsed thoroughly with water for 10 s, and gently air dried until a frosted appearance of the occlusal surface occurred. Subsequently,
Helioseal F was applied into the fissures with a tip syringe and spread with a dental probe to prevent air entrapment prior to polymerisation.

In Enamel Loc group, prior to application the occlusal surface of the teeth were air dried and the sealant material was applied onto pits and fissures; the tip was used to sweep on enamel and left undisturbed for 15 s before polymerisation.

In GC Fuji-VII capsule group, after the mixing of the capsules with the capsule mixer (GC Silver Mix 90) for 10 s, the mixture was extruded onto the tooth surface and a dental probe was used to spread a thin film of the sealant material into the pit and fissures before polymerisation.

All the sealant materials were light-cured for 20 s with LED (Bluephase C5, Ivoclar Vivadent) with an output of 500 mw/cm².

All teeth were treated by the same operator and stored in distilled water until the dye penetration procedure was performed.

Thermocycling and penetration
The samples were thermocycled in water for 500 cycles between 5 and 55°C with a dwell time of 30 s. The apices of teeth were covered with glass-ionomer cement and covered completely with nail varnish, except for the area within 2 mm of sealant varnish interface, and immersed in 1% methylene-blue for 24 h at 37°C.

Evaluation
After thermocycling and dye penetration, the molars were rinsed thoroughly with water and roots were removed using a diamond bur. After that, the nail varnish was removed from the teeth crowns. The sealed crowns were embedded in self-curing acrylic and sectioned with three parallel cuts in bucco-lingual direction through the mesial, central and distal fissures resulting in four molar fragments with six section sides available for inspection. Microleakage was scored by two independent observers using a light microscope with a magnification of X 40 (Leica Microsystems stereo microscope, Ltd. Stereo and microscope systems; Heerburg, Switzerland). Microleakage per section side was scored on a 3 point rating scale (score 0: no microleakage visible, score 1: microleakage up to half of the fissure, score 2: microleakage more than half of the fissure). In case of disagreement between two observers, a third independent observer was consulted to make the final decision. To determine the intra-examination reliability, 10 randomly selected molars were re-evaluated for microleakage.

The results were analysed using Kruskal Wallis test, while study groups were compared with Mann-Whitney test for statistically significant differences at 5% significance level.

Results
For microleakage measurements, Kappa statistics showed a perfect intra-examination reliability (K=1). Distribution of microleakage scores and the mean microleakage values are shown in Figure 1 and Table 2 respectively. In both contaminated and uncontaminated conditions, the number of samples showing no dye penetration (scored 0) was found mostly in Helioseal F group. The number of samples which showed microleakage more than half of the fissure (score 2) was frequently observed.
in Fuji VII/contaminated (Group VI) and Fuji VII/uncontaminated (Group III). No sealant loss was recorded during the study. In both absence and presence of saliva contamination, there was a statistically significant difference found among three pit and fissure sealant groups. Helioseal F showed statistically significantly less microleakage than Enamel Loc and Fuji VII, whereas no significant difference was noted between Enamel Loc and Fuji VII groups. Although the mean microleakage scores for Helioseal F and Enamel Loc were higher in saliva contaminated group in comparison to uncontaminated groups, the difference was not statistically significant. The mean microleakage score of Fuji VII remained the same for both uncontaminated and saliva contaminated group; p values between different sealant groups are given in Table 3.

## Discussion

In this study, microleakage of a conventional light-cured unfilled resin-based, a self-etching resin-based and a glass-ionomer fissure sealants were evaluated under uncontaminated and saliva contaminated conditions.

The long term clinical success of fissure sealants is closely related to their proper handling [Barroso, 2005]. A dry enamel surface is necessary to achieve good adhesion. Cotton-roll isolation requires four-handed dentistry; moreover, in these conditions contamination during swallowing and tongue movement is still possible [Tulunoglu et al., 2006; Grande et al., 2005; Mali et al., 2006]. Most of the marginal leakage test [Raskin et al., 2001; Amarente et al., 2003] revealed that there was no difference in microleakage between a glass-ionomer cement and a conventional light-cured unfilled resin [Ashwin, 2007]. Although the same glass ionomer cement, Fuji VII, was used in the study, the findings were indicating that it was comparatively similar to the conventional unfilled resins, with additional benefit of fluoride release. These differences might derive from the different design set up of each research or the evaluation method. The most common method to evaluate the interface between sealant materials and tooth structures is the marginal leakage test [Raskin et al., 2001; Amarente et al., 2006; Grande et al., 2005; Mali et al., 2006]. Most of the marginal leakage tests described consist of sectioning the dyed tooth at a number of places followed by measuring the extension of dye penetration in the interface. The comparison of the results is difficult because different dyes and different testing techniques have been applied over

### Table 2 - Mean microleakage scores of the sealant materials in uncontaminated and contaminated pit and fissure surfaces.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean microleakage score ± sd</th>
</tr>
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<tbody>
<tr>
<td>Group I: Helioseal F / uncontaminated</td>
<td>0.37 ± 0.61</td>
</tr>
<tr>
<td>Group II: Enamel Loc / uncontaminated</td>
<td>0.65 ± 0.69</td>
</tr>
<tr>
<td>Group III: Fuji VII / uncontaminated</td>
<td>0.90 ± 0.75</td>
</tr>
<tr>
<td>Group IV: Helioseal F / contaminated</td>
<td>0.33 ± 0.75</td>
</tr>
<tr>
<td>Group V: Enamel Loc / contaminated</td>
<td>0.77 ± 0.72</td>
</tr>
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### Table 3 - p values between different fissure sealant groups.

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<td>Group I</td>
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<td>p=0.00</td>
<td>p=0.24</td>
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<td>p=0.06</td>
<td>p=0.04</td>
</tr>
<tr>
<td>Group II</td>
<td>p=0.00</td>
<td>p=0.06</td>
<td>p=0.98</td>
<td>p=0.24</td>
<td>p=0.04</td>
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</tr>
<tr>
<td>Group III</td>
<td>p=0.37</td>
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<tr>
<td>Group IV</td>
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**SALIVA CONTAMINATION ON MICROLEAKAGE OF PIT AND FISSURE SEALANTS**

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**TABLE 3 - p values between different fissure sealant groups.**
the years [Raskin et al., 2001; Amarente et al., 2006]. A standardised method for measuring marginal leakage is required for accurate comparisons.

Based on our findings, Enamel Loc yielded higher microleakage scores compared to Helioseal F, whereas it performed similar to Fuji VII group under both contaminated and uncontaminated conditions. This might be due to the inadequate etching of the enamel compared to Helioseal F, since 37% phosphoric acid is applied prior to the application. By the time we started our study Enamel Loc was available on the market and was known as a relatively new self-etching sealant. However, at the time of the completion of the study, it was discontinued by the manufacturer due to inferior clinical results. It was claimed that temperature changes caused separation in the sealant material which can affect the chemical composition and clinical success of the material. Therefore, it is suggested that prior to launching new dental materials on the market sufficient clinical studies should be performed.

It is noteworthy to underline that microleakage represents only one aspect of the success of a sealant materials. Retention and bond strength of sealants are among other factors that affect microleakage. In cases where saliva contamination is unavoidable, the use of a bonding agent under sealant material is recommended to improve retention and reduce microleakage [Lussi and Duangthip, 2003; Feigal and Hebling, 2000; Hevinga et al., 2007]. On the contrary, some studies indicate that bonding does not affect retention and clinical success [Pinar et al., 2005; Tulunoglu et al., 1999].

Conclusion

New dental materials with simplified application steps are launched in the market. However it should be noted that this does not necessarily imply that they perform better under clinical conditions. Within the limitations of this in vitro study, it was concluded that, despite the fact that Helioseal F requires more application steps and it is more technique sensitive, it performed better than Enamel Loc and Fuji VII under both contaminated and uncontaminated conditions.

Clinical success of the pit and fissure sealant is material dependant however, optimum isolation is still the golden key for an effective seal. Besides in vitro studies, further clinical studies with longer observation period will be required to evaluate the success rates of the tested sealant materials.

References


