Original research:

Microleakage and depth of penetration of different materials used as pit and fissure sealants under wet conditions: An Invitro study

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Abstract

Objectives: To compare the effectiveness of 3 types of pit and fissure sealants namely, glass ionomer sealants (GC FUJI VII), high viscosity GIC used as ART sealant (GC Fuji IX EXTRA), and hydrophilic resin sealant (Embrace Wet Bond) when placed under salivary contamination in terms of marginal leakage and depth of penetration.

Methodology: Sixty extracted non-carious maxillary premolars were randomly divided into three groups of 20 each. Samples were prepared and sealant was applied as per the manufacturer’s instructions. Prior to sealant application the tooth surfaces were wetted with artificial saliva for 5 seconds using a micro brush applicator and air-thinned for 1 second. The samples underwent thermocycling followed by immersion in 5% methylene blue for 24 hours. The samples were sectioned buccolingually and analysed under a stereomicroscope to assess microleakage and depth of penetration. Mann-Whitney U test was applied to test the statistical significance of the difference in the mean values of microleakage and percentage penetration, with a p-value of <0.05 considered to be statistically significant.

Result: The hydrophilic resin sealant showed the least microleakage and greatest depth of penetration, with the differences in both parameters being statistically significant.

Conclusion: The hydrophilic resin sealant can be considered a promising material for sealing fissures, especially in very young children where isolation is difficult.

Keywords: Microleakage, penetration depth, Pit and fissure, Sealants

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Introduction

The use of caries preventive approaches such as professionally applied fluorides, community water fluoridation, plaque control measures, and sugar substitutes has led to a decline in the prevalence of dental caries in the Western world. However, this decrease in caries was found to be mostly on the smooth surfaces of teeth. [1, 2] Pit and fissure caries account for 80% of the total caries experience. [3, 4] The carious potential is directly related to the shape and depth of pits and fissures. It provides an optimal site for food lodgement and bacterial retention rendering mechanical means of debridement inaccessible. Such caries-susceptible sites should be sealed for effective caries prevention. Cariostatic properties of sealants are attributed to the physical obstruction of pits and grooves. Pit and fissure sealants are highly effective in reducing the rate of occlusal caries on permanent posterior teeth by forming a barrier between the tooth surface and the oral environment. [5] Even though the caries preventive benefits of pit and fissure sealants are well documented, they are still underused worldwide. One of the primary reasons for this is due to the technique-sensitive nature of its application. [6] For pit and fissure sealant application to be cost-effective, they should ideally be used on children at high risk of developing caries, and ideally, they should be applied on the pits and fissures of the teeth as soon as the tooth erupts.

Isolation of teeth is difficult in partially erupted molars and in young children. Most sealant materials used today are resin-based materials with high retention rates. But their use is clinically limited by the difficulties inherent in the use of resins in a moist environment. BisGMA-based materials are primarily hydrophobic in nature and require a dry field [7] Salivary contamination following etching is one of the main causes responsible for the failure of resin sealants. [8] To overcome the problem of technique sensitivity, various investigators have suggested using Glass ionomer sealants (GIS). Glass ionomers have the advantage of chemically bonding to the tooth, acting as a fluoride reservoir, and being more moisture tolerant as compared to Bis GMA resin [9] GIS includes low-viscosity GICs (Fuji VII) and high-viscosity GICs (Fuji IX GP) which have mostly been used as sealants in the atraumatic restorative treatment approach (ART sealants). [10] GIC sealants have been shown to have comparable caries preventive effects to resin-based sealants even though they have been shown to have lower retention rates. [11] More recently, hydrophilic resin sealant technology (EmbraceTMwetbondTM) has been developed with a moisture-tolerant resin-based sealant containing no BisGMA and no Bisphenol A and uses hydrophilic resin chemistry [7] As per the manufacturer, this sealant incorporates di-tri and multifunctional acrylate monomers into an advanced acid-integrating chemistry that is activated by moisture. Ideally, a pit and fissure sealant should be easy to apply, have good flow, good adaptation to the fissure walls, and have good abrasion and wear resistance. Most sealant materials have been studied under optimal conditions. Thus this study was done to assess and compare the effectiveness of hydrophilic resin sealants and GIS with respect to marginal microleakage and depth of penetration when used under conditions where there is salivary contamination.

Materials and Methods

Ethics Approval: The study was conducted in line with the principles of the Declaration of Helsinki. Ethical approval from the Institutional Ethics Committee (B2-25420/IEC-SSDC-10) was acquired before proceeding with the current study. This was an in-vitro study that included 60 intact maxillary premolars extracted for orthodontic purposes free of caries, restorations, fractures or any other defects.

Sample Preparation: The extracted teeth were thoroughly cleaned of soft tissue, debris and any deposits by washing under running tap water followed by ultrasonic scaling. The samples were then polished using pumice slurry and a prophylaxis cup and stored in normal saline at ambient temperature until further use. The samples were randomly allotted into 3 groups depending upon the type of sealant to be received.

Group 1: included 20 teeth for GIC sealant (GC FUJI VII CAPSULE) application

Group 2: included 20 teeth for ART sealant (GC FUJI IX EXTRA CAPSULE) application

Group 3: included 20 teeth for hydrophilic resin sealant (Embrace Wetbond) application

Prior to sealant application, the pits and fissures and adjacent enamel of the teeth in group 1 and group 2 were conditioned with GC Fuji cavity conditioner for 10 seconds rinsed, and dried, while the samples (surfaces to receive sealant) in group 3 were etched with 37% phosphoric acid for 15 seconds followed by rinsing and drying. No bonding agent was used. To simulate a wet environment, all the samples were wetted with artificial saliva for 5 seconds which was then air thinned for 1 second each of the sealants was applied as per the manufacturer’s instructions. In
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Group 1 GC Fuji VII was applied using a GC capsule Applicator. The material was extruded onto the tooth surface and manipulated into the pits and fissures using a micro brush. In group 2 GC Fuji IX Extra (Capsule) was extruded onto the pits and fissures from a GC Capsule applicator and pressed into the pits and fissures using a gloved index finger lubricated with petroleum jelly. The excess material was removed with a carver. In group 3, embrace wet bond was applied using the supplied applicator tip, and the material was further manipulated into the desired areas using a micro brush and light cured for 10 seconds.

The samples were then stored in distilled water for 24 hours, followed by thermocycling (250 cycles between 50°C & 55OC) with an immersion time of 30 seconds in each bath and a transfer time of 10 seconds. After thermocycling, a layer of sticky wax was applied at the apex and the surface of the teeth was painted with 2 layers of nail varnish leaving 2mm around the sealant border uncovered. The specimens were then immersed in 5% methylene blue for 24 hours and cleaned thoroughly under running tap water to remove excess dye. The teeth were sectioned bucco-lingually through the sealant using a straight handpiece with a diamond disc. The sectioned samples were examined under stereo microscope attached to a digital camera at a magnification of 10X and the photographs were taken.

**Table 1. Scoring criterion for microleakage is as follows**

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No dye penetration</td>
</tr>
<tr>
<td>1</td>
<td>Dye penetration restricted to outer half of enamel-sealant interface</td>
</tr>
<tr>
<td>2</td>
<td>Dye penetration in the inner half of enamel sealant interface</td>
</tr>
<tr>
<td>3</td>
<td>Dye penetration into the underlying fissure</td>
</tr>
</tbody>
</table>

The depth of penetration of sealant was analysed using the Image J software under 10x magnification, the penetration ability of sealant was calculated as a percentage of length A-B to length A-C, ie to obtain

\[
\frac{AB}{AC} \times 100
\]

**Statistical Analysis:** Statistical Analysis was done using SPSS version 20.00 (Chicago, USA). Categorical variables were represented using numbers and percentages while continuous variables were represented using mean ± standard deviation. The Mann-Whitney U test was applied to test the statistical significance of the difference in the mean values of micro-leakage and percentage penetration between groups. A P value of < 0.05 was considered to be statistically significant.

**Sample Evaluation**

Evaluation of dye penetration was done based on a 4-point scoring system given by Overbo & Raada (Table 1) by a single observer. [12]
Results

Microleakage Scores
All 20 samples in group 1 (GC Fuji VII CAPSULE) and group 2 (GC Fuji IX EXTRA) showed a score of 3 for microleakage, whereas, in group 3 (Embrace™ Wetbond™), 13 samples showed a score of 3, while the remaining 7 had a score 2. There was a statistically significant difference between group 3 and group 2, and group 3 and group 1 with respect to microleakage.

Figure 2. Micro leakage patterns in (A) GC Fuji VII CAPSULE, (B) GC Fuji IX EXTRA and (C) Embrace™ Wetbond™ groups.

There was a statistically significant difference between group 3 and group 2, and group 3 and group 1 concerning microleakage (Table 2).

Table 2: Distribution of microleakage score (0-3) (n=20) in three groups

<table>
<thead>
<tr>
<th>Groups n = 20</th>
<th>Score 0</th>
<th>Score 1</th>
<th>Score 2</th>
<th>Score 3</th>
<th>Mean +/- SD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (GC Fuji VII CAPSULE)</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>20</td>
<td>3 ±0</td>
</tr>
<tr>
<td>Group 2 (GC Fuji IX EXTRA CAPSULE)</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>20</td>
<td>3 ±0</td>
</tr>
<tr>
<td>Group 3 (Embrace™ Wetbond™)</td>
<td>Nil</td>
<td>Nil</td>
<td>7</td>
<td>30</td>
<td>2.65±0.48</td>
</tr>
</tbody>
</table>

*SD - Standard Deviation

Penetration Depth
The percentage penetration of sealants was calculated using ImageJ software (Table 3). Maximum penetration of sealant was observed in group 3 (92.53±7.625) followed by group 2 (81.7 ±11.08) and group 1 (73.68 ±11.95). The difference in penetration of sealant was found to be statistically significant between all the groups. (Table 4)

Table 3: Mean percentage penetration among different groups during the study

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean percentage penetration</th>
<th>Confidence interval (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>73.68 ±11.95</td>
<td>(68.45, 78.91)</td>
</tr>
<tr>
<td>Group 2</td>
<td>81.7 ±11.08</td>
<td>(76.85, 86.55)</td>
</tr>
<tr>
<td>Group 3</td>
<td>92.53 ±7.625</td>
<td>(89.19, 95.87)</td>
</tr>
</tbody>
</table>

Table 4: Comparison of percentage penetration between groups using Mann Whitney U test.

<table>
<thead>
<tr>
<th>Groups</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups 1 Group 2</td>
<td>0.042</td>
</tr>
<tr>
<td>Groups 1 Group 3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Groups 2 Group 3</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Discussion

This in vitro study was done to evaluate and compare the efficacy of three different types of sealant materials in terms of depth of penetration and microleakage placed under salivary contamination. The efficacy of pit and fissure sealants in preventing pit and fissure caries is well documented ever since its introduction in 1967. [13] A recent Cochrane review evaluating the caries-preventing effect of sealant in children and adolescents reported that resin-based sealants decreased caries increment by between 11 to 51% over a 2-year follow-up period. [14] For sealants to be most effective they should be applied to children in the ‘high-risk’ caries group and ideally should be applied as soon as the tooth emerges into the oral cavity. One of the main challenges faced in the placement of sealant, especially in children is obtaining adequate isolation. Exposure of the etched enamel to salivary proteins for even 0.5 seconds can affect resin sealant bonding and in turn sealant retention. [15] Under ideal conditions with optimal moisture control, resin-based sealants are the material of choice. However, these ideal conditions are rarely achievable in a young child.

To overcome this challenge glass ionomer cements have been advocated as pit and fissure sealants with some investigators recommending their use in clinical situations where isolation is difficult. GIC sealants are of two types namely the low viscosity and high viscosity types. Most of the earlier used GIC sealants were low-viscosity GICs (Fuji III GIC) which had poor physical properties. A newer generation GIC sealant is Fuji Triage (VII) (GC Tokyo, Japan) has improved physical properties and releases a higher amount of fluoride. [16] High Viscosity Glass Ionomer Cement (HVVIC) such as Ketac Molar Easymix (3M Espe Seefeld Germany) and Fuji IX (GC Tokyo, Japan) have been used as ART sealants which are applied in vulnerable pits and fissures using finger pressure.[10] A recent meta-analysis reported that the caries preventive effect of ART sealants was 97% after a follow-up period of 3 years with a survival rate of 72% suggesting that they would be a suitable alternative to Resin Based Sealants. [17] Also, alternative techniques such as applying an intermediate layer of bonding agent have been recommended to improve bonding to the contaminated tooth surface.[18] However, this technique is not popular probably due to the additional step required and lack of cost-effectiveness. More recently hydrophilic resin-based pit and fissure sealants have been developed to overcome the challenge of sealing pits and fissures under moisture or salivary contamination. [9] EmbraceTM WetbondTM is the first pit and fissure sealant that bonds to the moist tooth. Research shows remarkable sealing ability and adaptation to the tooth structure. [19]

To the best of our knowledge, there are no studies comparing the performance of GIC sealants and hydrophilic resin sealants under moisture or salivary contamination. Previous studies have compared Resin Based Sealants to hydrophilic sealants and GIC sealants to Resin Based Sealants/ hydrophilic sealants mostly under ideal conditions. [8]

In the present study, upper first premolars were chosen as these teeth were less likely to show variation in pit and fissure morphology as compared to molars [20] The samples in all the groups were prepared as per the manufacturer’s instructions. All samples were subjected to thermocycling to mimic the stresses the sealants would be subjected to in the oral environment. The temperature was set between 5°C and 55°C incorporating 250 cycles as proposed by Butail et al. [21]

In our study microleakage was assessed using 5% methylene blue as this dye enables easy visualization and also provides excellent contrast with the surrounding environment thus enabling easy scoring. [22] In the present study, the hydrophilic resin sealant performed better in terms of microleakage and depth of penetration as compared to GIC sealants Fuji VII and Fuji IX GP (ART sealant). Antonson SA et al in a Randomised Controlled Trial, compared the efficacy of GIC sealants and resin-based sealants in partially erupted molars and concluded that GIC sealants may be a better material for sealing partially erupted molars, as even though retention rates were similar at the 24 months, marginal discoloration was less in the GIC group with no caries. [23]

In a study comparing hydrophilic resin sealants and conventional resin-based pit and fissure sealants, Khogli et al, concluded that the hydrophilic resin sealant placed under moisture or salivary contamination showed similar microleakage and penetration to conventional sealants placed under optimal conditions. [24] As per the present study, all three groups showed some degree of dye penetration with all the samples in both the GIC sealant groups showing maximum dye penetration. Various investigations have suggested that microleakage can be expected in all restorative materials, however, the poor performance of GIC sealants as compared to the hydrophilic resin-based sealant in the present study could be due to the solubility and disintegration of the material in the water when subjected to thermocycling. [38, 39]

The penetration of sealants into the fissures is influenced by various factors like fissure morphology, preparation, acid etching of enamel surface, adhesive application, and contamination of fissure surfaces. In terms of penetration of sealant, Fuji VII was found to
Penetrate to a lesser depth than both Fuji IX and hydrophilic sealant. This is in contrast to the findings of Khanal et al, who compared the penetration depth of Fuji VII to conventional sealant under optimal conditions and found that GIC sealant performed better. This difference could be because, in the present study, no finger pressure was applied during the application of Fuji VII, which could have limited the adaptation and flow into the fissures. This could also be the reason why Fuji IX performed better than Fuji VII in this aspect. One of the limitations of the present study is that the samples were split buccolingually into two halves and only one half was used for the study. Whether examining multiple sections of a single sample would alter the outcome needs further investigation. Also, whether the superiority exhibited by hydrophilic resin sealants as compared to GIC sealants in terms of microleakage and depth of penetration as exhibited in this study makes them more effective pit and fissure sealants under clinical conditions needs further investigation.

Conclusion

Under the present in vitro study conditions, all the 3 sealant groups showed micro leakage with the hydrophilic resin sealant showing significantly less microleakage than both GIC sealant groups. The hydrophilic resin sealant showed a greater depth of penetration than both GC Fuji IX EXTRA and GC Fuji VII sealant groups. Thus we can conclude that in conditions where ideal isolation is not possible, hydrophilic resin sealants may be considered for use as pit and fissure sealants.

References


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