ORIGINAL ARTICLE

Evaluation of Marginal Microleakage and Depth of Penetration of Different Materials Used as Pit and Fissure Sealants: An *In Vitro* Study

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Abstract

Objective: Fissure sealants hold a great significance in the preclusion of inception of caries process. The present *in vitro* study assesses the marginal sealing ability and penetration depth of various dental products used as pit and fissure sealants.

Study design: Sixty freshly extracted human non-carious premolars were arbitrarily categorized into four groups of 15 samples. Prophylaxis of occlusal surfaces of sample teeth was done with pumice slurry and sealant was applied. Later, the teeth underwent thermocycling and immersion in 5% methylene blue for 24 hours. Sectioning of teeth samples was done buccolingually and they were analyzed under stereomicroscope.

Statistical analysis used: Nonparametric tests Kruskal–Wallis and Mann–Whitney were applied to carry out microleakage comparison. The percentage penetration depth was compared using the one-way analysis of variance (ANOVA) test. Tukey's *post hoc* test was applied for multiple analogies.

Results: Highest microleakage was seen in glass ionomer-based sealant followed by flowable composite and least for classical sealant. Flowable nanocomposite gave comparable results with that of the classical sealant. No statistical difference was found with respect to depth of penetration between different tested materials.

Conclusion: Flowable nanocomposite can be considered as a promising substitute for sealing fissures and thus can be endorsed to caries-susceptible pediatric patients.

Keywords: Microleakage, Penetration depth, Pit and fissure, Sealants.

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Introduction

"An ounce of prevention is worth a pound of cure."

The shape and depth of occlusal pits and fissures provide an optimal site for food lodgment and bacterial retention rendering mechanical means of debridement inaccessible.[1] Such caries-susceptible sites should be sealed for an effective caries prevention.[2] Occlusal sealing reduces risk of caries to a greater extent compared with the teeth which are not sealed, as well as sealant placement is cost-effective when compared with cements used for restoring cavities.[3] The intent of study was to probe efficacy of various materials used as sealants, by evaluating marginal microleakage and penetration depth of materials into occlusal pits and fissures.

Materials and Methods

Sixty intact maxillary/mandibular premolar teeth extracted for orthodontic reasons without any carious lesion were included in the study. After collection of teeth samples, saliva and blood were cleaned and brushed. Extraneous soft tissue, superficial debris, and calculus were removed from the teeth with an ultrasonic scaler. Pretreatment of sample teeth was done by giving a prophylaxis with an aqueous pumice slurry, using a prophy cup. Later, samples were washed thoroughly with water and dried. The samples were kept in normal saline at ambient temperature to prevent them from becoming brittle because of dehydration.

The samples were arbitrarily categorized into four groups of 15 teeth:

Group I: Fifteen teeth specimens to be sealed with classical pit fissure sealant (Helioseal F; Ivoclar Vivadent AG, Schaan, Liechtenstein).

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Group II: Fifteen teeth specimens for application of flowable composite (Te-Econom Flow; Ivoclar Vivadent AG).

Group III: Fifteen teeth specimens for application of flowable nanocomposite (Tetric N-Flow; Ivoclar Vivadent AG).

Group IV: Fifteen teeth specimens to be sealed with glass ionomer-based sealant (Fuji VII; GC Corporation, Tokyo, Japan).

Sealant Application

The occlusal enamel surface of samples in the first three groups was etched using 37% phosphoric acid for 30 seconds. The etched surface was rinsed and air-dried. Sealant application was done on fissures as per the manufacturer's guidelines. The tip of periodontal probe (API) was gently moved through the fissure to prevent voids and air entrapment. Later, a light cure unit was used on the occlusal surfaces for polymerization. Prior to the application of sealant, bonding agent was applied to the fissures only in group II and group III using a microbrush and was polymerized for 10 seconds (according to the manufacturer's guidelines). In group IV, mixing of the sealant material was done according to the manufacturer's guidelines. Mixed cement was applied into pits and fissures using a plastic filling instrument and was cured for 20 seconds. A coat of Vaseline was applied over the sealant with a gentle pressure on the specimen.

Thermocycling and Dye Immersion

The four groups were kept in distilled water for 24 hours at ambient temperature after sealant placement. Thermocycling of samples was carried out (250 cycles between 5°C and 55°C), with an immersion time of 30 seconds in each bath and a transfer time of 10 seconds. A layer of sticky wax was applied at the apex of each sample after thermocycling. The surfaces of teeth were painted with two layers of nail varnish leaving 2 mm around sealant borders. Specimens were immersed in 5% methylene blue for 24 hours. Teeth samples were cleaned under water to remove excess dye and were sectioned buccolingually through the sealant using a high-speed straight handpiece, diamond disk, and water spray. Sectioned samples were examined under stereomicroscope at a magnification of 10× and photographs were taken.

The assessment process was conducted on the basis of 4-point scoring system by a single observer using Ovrebo and Raadal[4] criteria for evaluating dye penetration.

Criteria for Grading Microleakage[4]

Score 0: No dye penetration (Fig. 1)

Score 1: Dye penetration restricted to outer half of enamel–sealant interface (Fig. 2).

Score 2: Dye penetration in inner half of enamel–sealant interface (Fig. 3).

Score 3: Dye penetration into underlying fissure (Fig. 4).

The images were analyzed using the CorelDRAW software under 100% magnification. Measurement tools in the software were used for calculating the penetration of the sealants within the fissure system. To denote the top of the fissure (A), upper meniscus of sealant was used. Sealant penetration ability was calculated as a percentage of length A–B to length A–C, i.e., the length of the central groove filled with sealing material was divided by the measurement corresponding to its total depth to obtain the percentage of sealing of the occlusal groove[5] as shown in Figure 5.

Data management and analysis was done. Nonparametric tests Kruskal–Wallis and Mann–Whitney were applied to carry out microleakage comparison between sealants. The percentage penetration depth was compared using the one-way ANOVA test. Tukey's *post hoc* test was used for multiple analogies. A *p* value of less than 0.05 was contemplated to be statistically significant.

Results

Microleakage and penetration depth were observed in all groups.

Microleakage

No sealant was lost. All the groups (I–IV) showed microleakage of sealants. The microleakage scoring is depicted in Table 1.

The mean microleakage score was highest in Fuji VII (group IV). According to Mann–Whitney test, statistically remarkable variance was present among all study groups with respect to mean microleakage except between group I and group III (Table 2).

Penetration Depth

The percentage penetration of sealants was calculated using CorelDRAW Image analysis software. The mean observations calculated are shown in Table 3. Mean percentage penetration was recorded maximum in group I (86.24 ± 7.80), followed by group III (84.88 ± 10.00), then group IV (83.80 ± 8.03), and minimum in group II (78.56 ± 12.50). Intergroup comparison revealed no statistically significant difference in mean percentage penetration of different sealant materials.

Discussion

Favorable marginal adaptability of a sealant to enamel influences its efficacy and thus minimizes microleakage. In our study, pretreatment prophylaxis with pumice and etching of samples with acid was opted because most dentists validate these steps for sealant application and are also advocated by the manufacturer.[6] Ansari et al.[7] proposed prophylaxis with pumice enhances sealant penetration and adaptation and reduces microleakage. The different sealants used in the present study were applied without enameloplasty, as done in the *in vitro* study by Prabhakar et al.[8] and Bahrololoomi et al.[6]

Fig. 1: Photograph of sample showing score 0

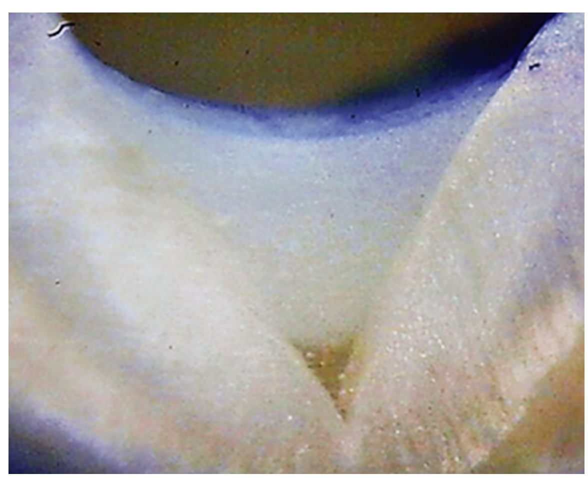

Fig. 2: Photograph of sample showing score 1

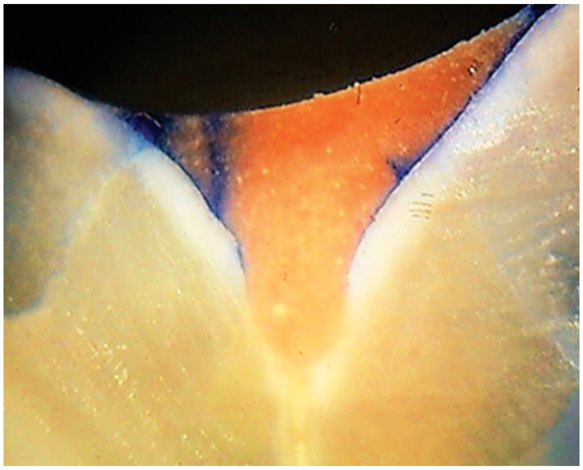

Fig. 3: Photograph of sample showing score 2

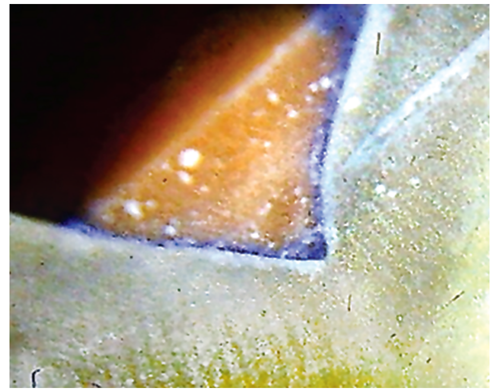

Fig. 4: Photograph of sample showing score 3

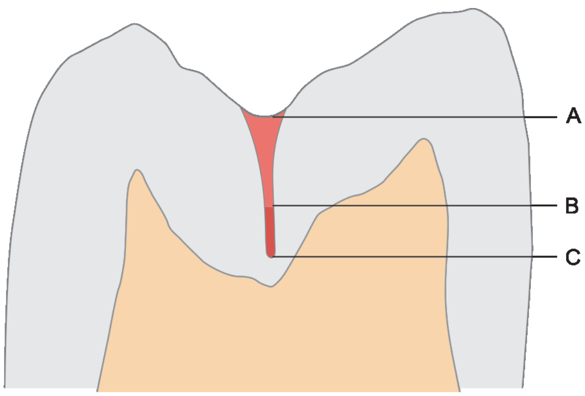

Fig. 5: Points of reference used to determine depth of fissure and percentage sealant penetration

In the present study, acid etching for 30 seconds was done in group I, group II, and group III to enhance sealant bonding. Etchant application results in roughness of tooth surface, thereby forming a honeycomb-like structure which accelerates the sealant penetration into the enamel, thus retaining the sealant.[9] Acid etching was followed by application of dentin bonding adhesive only in two groups, i.e., group II and group III, which were polymerized for 10 seconds. Bahrololoomi et al.[6] and Dukic and Glavina[10] showed that dentin bonding adhesive application prior to sealant placement did not alter microleakage significantly.

The outcome of resin materials in an *in vitro* study can be examined by thermocycling which is used to duplicate the long-standing stresses to which the restorations are exposed.[11] In our study, the temperature was set between 5°C and 55°C, as asserted by several studies done by Penugonda et al.[12] and Styner et al.[13] Thermocycling incorporating 250 cycles approved by Smith et al.[14] was carried with a dwell time of 30 seconds as suggested by Bullard and Leinfelder.[15] No considerable changes in microleakage between 250 and 500 cycles were noted by Smith et al.[14]

In our study, microleakage assessment was done using 5% methylene blue dye. Methylene blue dye penetration method imparts perfect and easy visualization in the digital images for scoring with a clear reference point, thereby providing excellent contrast with the surrounding environment.[16] As suggested by Hatibovic-Kofman et al.[17] and Birkenfeld et al.,[18] the study specimens were stored for 24 hours in methylene blue.

The sections were observed under stereomicroscope which was attached to a digital camera with the help of dye and CorelDRAW software. Pardi et al. adopted a similar technique for observation.[19]

Our study results showed that all the groups exhibited some degree of dye penetration. This finding is in accordance with those reported by Theodoridou-Pahini et al.[20] and do Rego and de Araujo[21] who stated that microleakage can be expected in all restorative materials. This may be because the coefficient of thermal expansion of sealants is much greater than the coefficient of thermal expansion of the teeth. In our study, classical sealant depicted less microleakage than flowable composite. Francescut and Lussi,[22] who did microleakage and penetration depth assessment between flowable composite and conventional sealant, support the present study findings. Kwon and Park[23] found that no voids were observed in conventional sealants, whereas flowable composites resulted in voids due to higher viscosity.[23] Classical sealants such as Fissurit F have remarkably less microleakage than flowable composites as suggested by Duangthip and Lussi.[24] No difference in microleakage using flowable composite and hybrid composite was reported by Gillet et al. in their study which is in contradiction to the findings of our study.[25]

Results derived in the present study showed highest degree of microleakage in group IV using Fuji VII glass ionomer sealant when compared with the other three groups. Higher degree of dye penetration in glass ionomer cement (GIC) when compared with resins has been reported by Kidd.[26]

Dhar and Tandon stated that greater gaps exist between the tooth and the sealant at the interface in glass ionomer cements than resin-based sealants.[27] Our finding is in line with do Rego and de Araujo's study in which resin-modified glass ionomer had greater microleakage scores compared with resin-filled sealant.[21] Likewise, in a study by Ganesh and Tandon, the resin-based sealant group performed much better with less microleakage than did the Fuji VII group.[28] However, in contrast to our findings, a study by Ashwin and Arathi revealed that there was no variance in microleakage between Fuji VII (GIC) and 3M Concise (conventional light-cured unfilled resin).[29] Pardi et al. showed no differences between resin-based, resin modified glass ionomer cement (RMGIC), and conventional glass ionomer sealants.[19]

Our study results were in accordance with Singh et al.'s study who concluded that percolation of three various materials used as sealants was highest for flowable composite and least for conventional sealant. The values for nanocomposite group were intermediary.[30]

Table 1: Distribution of microleakage score (0–3) ($n = 15$) in four groups

n = 15	*Score 0*	*Score 1*	*Score 2*	*Score 3*	*Mean ± SD*
Group I (Helioseal F)	13	1	1	0	0.20 ± 0.561
Group II (Te-Econom Flow)	3	10	2	0	0.93 ± 0.594
Group III (Tetric N-Flow)	12	2	1	0	0.27 ± 0.594
Group IV (Fuji VII)	1	1	9	4	2.07 ± 0.799

SD, standard deviation

Table 2: Showing comparison of microleakage between groups using Mann–Whitney test

Groups	*p value*	*Inference*
I and II	0.001	Significant
I and III	0.654	Not significant
IV and I	<0.001	Significant
II and III	0.003	Significant
IV and II	<0.001	Significant
IV and III	<0.001	Significant

Table 3: Showing mean percentage penetration among different groups during the study

Groups	*Mean percentage penetration*
Group I	86.24 ± 7.80
Group II	78.56 ± 12.50
Group III	84.88 ± 10.00
Group IV	83.80 ± 8.03

The penetration depth is also a very major criterion which may affect the sealant retention. The penetration ability of pit and fissure sealants is influenced by several factors such as shape and morphology of fissures as well as properties of materials used.[25]

In our study, although classical sealant showed maximum penetration than other materials used, there was no statistically noteworthy variance ($p > 0.05$) among different groups with respect to depth of penetration. Our results were in accordance with Autio-Gold[31] and Duangthip and Lussi[24] and can be explicated by polymerization shrinkage of materials, which is associated with features like adhesion quality, viscoelastic properties, and curing process of materials.

The results of our study were consistent with Khogli et al.[32] who found no significant difference in penetration depth between the unfilled sealant (Delton) and filled sealant (Embrace). In addition, another *in vitro* study by Aguilar et al. compared (FluroShield) resin-based sealant with (Tetric Flow Chroma) flowable composite resin and observed similar penetration of the materials on the occlusal central groove.[3] Xalabarde et al. observed no variability in ingress or adaptation of unfilled and filled sealant materials.[33]

One of the constraints was the fact that our study was an *in vitro* evaluation. Fissure sealants might function distinctively depending on the environment due to several factors which include fissure morphology and preparation, acid etching of enamel surface, adhesive application, and contamination of fissure surfaces. Therefore, it is suggested that similar studies with saliva contamination be performed. Further *in vivo* studies, other flowable restoration systems, and different preparation methods should be carried out to clarify the exact clinical contribution of each of these factors. In this context, variables such as long-standing adaptation and shear bond strength of the materials used as sealants must be considered.

Conclusion

- None of the tested material could prevent dye penetration.
- Microleakage of different materials used in the study was least for classical sealant (Helioseal F) and highest for glass ionomer-based sealant (Fuji VII). Statistically considerable variance was found between different study groups with respect to mean microleakage except classical sealant and flowable nanocomposite (i.e., group I and group III).
- All the sealant materials presented similar penetration into the occlusal fissures as no statistically significant difference ($p > 0.05$) among the study groups was observed with respect to penetration depth.

The present study concluded that classical sealant was best in terms of both microleakage and penetration depth but as flowable nanocomposite gave comparable results with those of classical sealant, the use of flowable nanocomposite can be endorsed to caries-susceptible pediatric patients, as pit and fissure sealant.

Why this paper is important to pediatric dentist?

- Sealant application can serve as a tool in caries preventive programs.
- Noninvasive procedure with simple armamentarium.
- Newer materials enhance the efficacy of preventive procedure.

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