Microleakage and SEM Analysis of Flowable Resin Used as a Sealant Following Three Fissure Preparation Techniques – An *in vitro* Study

Chaitra TR * / Subba Reddy VV ** / Devarasa.GM *** / Ravishankar TL ****

Objective: *Preventive procedures using pit and fissure sealants are one of the important aspects of pediatric dental practice. The objectives of this in vitro study were to comparatively evaluate microleakage and resin tag penetration of a flowable resin used as a sealant on molars after preparation with Conventional, Enameloplasty and Fissurotomy techniques.* ***Method****: A total of 48 teeth were divided into 3 groups (n-16). Group A(CST),Group B (EST),and Group C (FT). Following the fissure preparation sealant was applied as per manufacturer's instructions. 8 samples in each group were used for Microleakage and Resin tag analysis. Microleakage analysis was done under Stereomicroscope after Methylene blue dye immersion by using scoring system. The resin tag analysis was done by measuring the length of resin tags on scanning electron microscope (SEM) images consisting of 10µm scale taken under optical zoom of 2000 X.* ***Results****: Significantly lesser microleakage score (P<0.01) and longer resin tag penetration (P<0.001) observed in EST (mean score 0.5±0.53) & (12.19±1.93µm) when compared to CST(mean score 1.75±0.89) & (5.96±1.84µm) and FT (mean score 1.5±0.53) & (6.76±1.82µm) which showed more microleakage and short resin tags. No statistical significance between CST and FT was found.* ***Conclusion****: EST has proven to be an excellent method for preparation of pits and fissures when Flowable composite is used as a sealant because of its lesser microleakage and longer resin tag penetration.*

Keywords*: Conventional Sealant technique (CST), Enameloplasty Sealant technique (EST), Fissurotomy technique (FT), Microleakage, Resin tag penetration.*

J Clin Pediatr Dent 35(3): 277–282, 2011

INTRODUCTION

Natural enamel and dentin are the best "dental materials" in existence and hence restorative techniques that conserve a greater part of the natural, healthy tooth structure must be considered desirable.[1] Pit and fissures are recognized as being highly susceptible to caries.[2,3] Factors responsible for the high incidence of occlusal caries include the lack of salivary access to the fissures as a result of surface tension, effectively preventing remineralization and reducing the effectiveness of fluoride.[2] Sealing pits and fissures in teeth is a widely advocated preventive technique.[4,5] A nagging issue is identification of the ideal technique for placement of sealants to maximize retention and prevent microleakage. Recently, alternative methods such as bur preparation and air abrasion have been proposed for sealant application as their efficacy in cleaning the pit and fissures of debris is better.[6] Enameloplasty or fissure enlargement with a bur has been advocated as a technique that enhances retention by allowing deeper penetration of etchant and sealant and increasing surface area for bonding.[6] It has been recommended in narrow deep pits and fissures in which caries might develop should be treated with an invasive pit and fissure sealing technique, as microbiologically this is the most rational of the invasive methods. In addition, it has been demonstrated that mechanical enlargement of occlusal fissures using the enameloplasty sealant technique allowed a superior sealant adaptation than conventional sealant techniques.[2] The applicability of flowable restorative systems in dentistry has increased mainly because of their beneficial properties, such as low viscosity, low modulus of elasticity and ease of handling. Flowable composite materials have better abrasion resistance and thus, provide a better retention than a conventional unfilled resin.[7, 8]

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Studies are being done to evaluate the effect of the additional techniques like fissurotomy and enameloplasty and materials like newer bonding agents that make the pits and fissures conducive to receive a flowable resin. These enhance the retention rate and reduce microleakage.[6, 7] The aim of the study was to compare and evaluate the microleakage and resin tag penetration of a flowable composite used as a sealant after preparation of pits and fissures, with conventional, enameloplasty and fissurotomy techniques.

MATERIALS AND METHOD

A total of forty-eight permanent mandibular third molars having deep pits and fissures were used in the study. The samples were thoroughly cleaned using scalers and careful visual inspection was carried out under good illumination on the clean dry surfaces and was palpated using explorer for caries free deep pits and fissures. Samples were stored in distilled water until experiment time. The teeth were randomly distributed into 3 groups (n=16).

Group A: Conventional technique was followed which consisted of Pumice prophylaxis followed by rinsing, drying, acid etching for 40 sec with 37% phosphoric acid and finally followed again by rinsing and drying.

Group B: Enameloplasty Technique was done by opening the pits and fissures using 1/4 round tungsten carbide bur under low speed,[9] followed by acid etching for 40 sec with 37% phosphoric acid and finally rinsing and drying.

Group C: Fissurotomy Technique was employed where Fissurotomy bur (micro short tapered fissure, SS WHITE, USA) was used to open up the fissures. The pits and fissures were prepared to the size of the bur head (1.5mm deep and 1/8 -1/10 inter cuspal distance)[1] after which acid etching was done for 40 sec with 37% phosphoric acid followed by rinsing and drying.

After the tooth preparation, all the groups were bonded with Tetric-N-Bond (Ivoclar Vivadent) adhesive and cured for 20 sec, which was followed by placement of flowable composite (Filtek Z350) as a sealant as per manufacturer's instructions. Out of 16 teeth in each group, 8 teeth were assessed for microleakage analysis and 8 teeth for resin tag analysis.

Microleakage analysis

Roots of the molar samples of all the groups were cut from crowns using a high speed water cooled diamond blade. The samples were placed in distilled water at 37º C for 24 hrs and then, samples were subjected to thermocycling for 200 cycles between 2º C and 58º C with dwell time of 15 seconds and a 15 second transfer time to simulate temperature fluctuations found in the oral cavity. The samples were coated with a nail varnish on the tooth surfaces, except on the restoration and 1mm rim of tooth structure around the restoration (Window) and were immersed in 2% Methylene blue dye for 48 hrs. After rinsing in tap water, samples were mounted mesio-distally on the acrylic block measuring 1inch × 1inch, exposing half the tooth structure. The teeth were sectioned labio-lingually across the center of the tooth using Hard Tissue Microtome.

The samples were then examined under x16 stereomicroscope magnification to analyze dye penetration at the marginal seal of the flowable composite.

Scoring criteria: Grande et al[10]

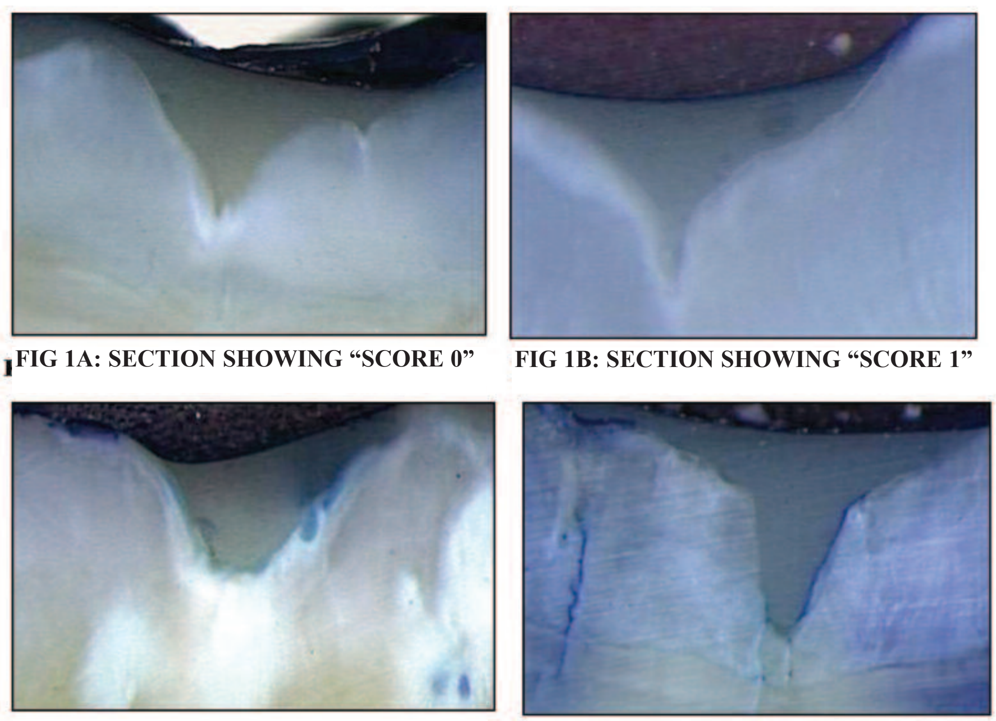

FIG 1A: SECTION SHOWING "SCORE 0"
FIG 1B: SECTION SHOWING "SCORE 1"
FIG 1C: SECTION SHOWING "SCORE 2"
FIG 1D: SECTION SHOWING "SCORE 3"

Figure 1. Microleakage Scores

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0 – No dye penetration. (Fig. 1A)
1 – Dye penetration up to 1/3 of the depth of fissure. (Occlusal third) (Fig. 1B)
2 – Dye penetration more than 1/3 and less than 2/3 of the depth of fissure. (Middle third) (Fig. 1C)
3 – Dye penetration more than 2/3 of the depth of fissure. (Apical third) (Fig. 1D)

Resin tag analysis

The resin tag analysis was done using Scanning Electron Microscope. The Crowns were sectioned to a thickness of 1 mm labiolingually using hard tissue microtome. Twenty four specimens belonging to 3 groups were cleaned in distilled water with ultrasonic agitation for 30 minutes and gently air dried. They were fixed to SEM stubs, gold sputtering was done for 10 min and the resin and tooth interface of the specimens were examined under SEM machine. The resin tag analysis was done by measuring the depth of the penetration of the resin tags in micrometers on scanning electron microscope (SEM) images consisting of 10µm scale, taken under optical zoom of 2000X. Mean length of resin tags in micrometer (µm) was calculated for all the three groups from a set of SEM images. From each image a mean of three longest resin tags was considered.

Statistical analysis:

The SPSS 11.0 and Systat 8.0 Statistical software were used for the analysis. For microleakage analysis, non-parametric test Kruskal-Wallis Anova by Ranks was used for multiple group comparisons and Mann Whitney U test for group wise comparisons. Resin tag was analyzed using One way ANOVA for multiple group comparisons followed by Students Newman kuel test for group wise comparison. Normality of the resin tag data was assessed using Shapiro –Wilk test. Results are expressed as Mean ± SD. For all the tests, a P-value of 0.05 or less was used for statistical significance.

RESULTS

Microleakage group

Table 1 shows the distribution and comparison of microleakage scores of all the three groups. Group A (CST) had the highest mean score (1.75±0.89) and Group B (EST) the least mean score (0.5± 0.53). Kruskal Wallis Anova Test analysis showed a significant difference between the groups ($p< 0.01$). It was observed there was a significant difference between Group A (CST) and Group B (EST) and also between Group B (EST) and Group C (FT), but no significant difference between Group A (CST) and Group C (FT). Since the microleakage was assessed on ordinal rating scores, non parametric test like Kruskal Wallis Anova by ranks was used.

Resin tag group

Table 2 shows the depth of resin tag penetration in different groups. Largest mean depth of resin tags penetration of 12.19±1.93µm was seen in Group B(EST) followed by 6.76±1.82 µm in Group C(FT) and 5.96±1.84µm in Group A (CST). Statistical analysis using One way ANOVA indicated a highly significant ($p<0.001$) difference between the groups. This test was used because of the continuous nature of the variable (resin tags in micrometers). Post-hoc analysis done using Student Newman Keul test among the three groups indicated a significant difference between Group A (CST) and Group B (EST) and also between Group B (EST) and Group C (FT) but there was no significant difference between Group A (CST) and Group C (FT) as shown in Table 2.

DISCUSSION

Fissure sealing or use of fissure sealants with unfilled resin or resin composites after acid etching of the enamel has been advocated as a reliable method for prevention of fissure caries. Because of the masticatory status of molar a more wear resistant material such as flowable composite is necessary rather than unfilled resin.[11] Excellent retention and longevity of sealants depend upon 3 factors, namely penetrability of the acid etched enamel, marginal sealing and wear/abrasion resistance.[12]

Microleakage Group

The clinically undetectable passage of bacteria, fluids, molecules, or ions between the cavity wall and the applied restorative material, is known as microleakage. In case of pit and fissure sealants, the success of this technique can be hindered if the applied material cannot resist microleakage, resulting in the initiation and / or progression of caries under sealed surfaces, as well as increasing the difficulty of diagnosing and treating this lesion. The quest for a perfect sealant is a real challenge and although bond strength is

Table 1. Comparison Of Microleakage Scores Observed In Different Groups

Sl No	Study Group	Range	Mean Microleakage	P-Value*	Significant Pairs**
1	GROUP A (CST)	001-3	1.75 ± 0.89	P<0.01 Significant	1&2, 2&3
2	GROUP B (EST)	0-1	0.5 ± 0.53		
3	GROUP C (FT)	001-2	1.5 ± 0.53		

* Kruskal Wallis Test
** Mann Whitney U Test

Table 2. Comparison Of Resin Tag Penetration Observed In Different Groupes

Sl No	Study Group	Range (µm)	Mean Penetration(µm)	P-Value*	Significant Pairs**
1	GROUP A (CST)	3.8-8.82	5.96 ± 1.84	P<0.001 Highly Significant	1&2, 2&3
2	GROUP B (EST)	9.09-15.15	12.19 ± 1.93		
3	GROUP C (FT)	2.42-8.0	6.76 ± 1.82		

* Oneway Anova
** Student Newman Keul Test

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important, microleakage is the major factor that affects the sealant efficiency.[13] Various dyes such as India Ink, Methylene blue, Basic Fuschin, and silver nitrate can be used to detect microleakage. Although silver nitrate is a superior dye because of its smaller particle size (0.059nm),[13] 2% Methylene blue was used in our study because it is equally efficient, easily available and convenient.

In the present study, comparison was done between 3 fissure preparation techniques. Results showed a greater microleakage in Conventional sealant technique (CST) group compared to Enameloplasty sealant technique (EST).The possible explanation for the greater microleakage in pumice prophylaxis is that, a rubber cup or pointed bristle brush with pumice, did not adequately clean pits and fissures to allow the etchant to produce a surface area receptive for bonding when compared to other methods which increased the surface area thus increasing the adaptability of the sealant.[14] Garcia-Godoy and Medlock showed that from the micro morphological stand point, prophylaxis with pumice and a pointed bristle brush or rubber cup is less effective than other preparation methods.[15]

The probable reason for decreased microleakage in EST is that, by enlarging the narrow fissures, the sealant easily penetrates and also it eliminates the acquired pellicle thus increasing the sealant adaptability. Hatibovic *et al* compared fissure preparation with different methods and reported that bur preparation with ¼ round bur coupled with acid etching to be significantly better at reducing microleakage when compared to prophylaxis and acid etching.[16] Geiger *et al* demonstrated that the deeper the level of sealant penetration, lower the probability of microleakage and enameloplasty provided deeper sealant penetration and less microleakage.[17]

The other fissure preparation technique which was used in this study was the Fissurotomy technique (FT). Results showed that significantly more microleakage occurred in this group when compared to EST but was similar to CST.

Freedman *et al* reviewed ultraconservative resin restorations and reported that the typical fissurotomy preparation is very narrow, long and irregularly deep and it is important that the restorative material should flow easily into all the nooks and crannies. The material of choice is a flowable composite, but in very deep fissures significant microleakage was observed.[1]

It has been observed in the present study that, some amount of microleakage was found in all the groups, lesser in enameloplasty and significantly more in conventional and fissurotomy techniques. This can be attributed to the property of the flowable composite which shows some amount of polymerization shrinkage following light curing, that may create microgaps between tooth and flowable composite which contributed to microleakage.[18] The other reason for the presence of microleakage in all the groups could be the process of thermocycling, where in, the samples were tempered at temperatures of 2º C and 58º C to simulate the oral conditions. It must be pointed out that in an oral cavity, besides the thermal changes, numerous factors like hydrolytic enzyme action, ionic composition of the saliva, pH fluctuation due to cariogenic microorganisms or acidic food may influence the quality of marginal integrity. With scanty data from the previous literature, pH cycling has shown no significant effects on microleakage,[19] so pH cycling was not done in this study.

Theodoridou-Pahini *et al* reported that, increased degree of microleakage occurs in the thermocycled specimens compared with those of non-thermocycled specimens and also reported that the thermal expansion coefficient of the sealants is significantly different from that of the enamel and the mechanics of expansion and contraction of the teeth are different from those of materials, thus affecting microleakage.[20]

Resin tag group

The resin sealant creates a mechanical bond with the underlying etched enamel rods by flowing into the micro spaces and forming resin tags but the pattern of the etched enamel may be different in each tooth as well as on the different surfaces of the same tooth. The resin tag penetration also is affected by the methods of preparation of the pits and fissures.[21] CST showed very short resin tags when compared to EST, which showed longer resin tags (Figs. 2 and 3). Garcia-Godoy *et al* observed rubber cup or bristle brush pumice prophylaxis does not completely remove material deposits from pits and fissures. In some cases, even after acid etching the enamel, the residual material that occludes the pits and fissures remains. The effect of the prophylaxis and acid etching was limited to the inclined cuspal planes. Moreover, the use of pumice as a prophylaxis agent could prevent the acid and sealant penetration into the pits and fissures. This was confirmed by the absence of tags and the presence of a smooth surface in the deeper portions of the sealant fitting surface.[22]

Loyola *et al* compared and evaluated the CST and EST using a flowable resin as a sealant in molars affected by dental fluorosis and reported that in CST, sealant did not flow into the bottom of the fissures leaving spaces that can favor the fracture of the material and initiate the process of dental caries. Minimal resin tag penetration was seen in CST[11]

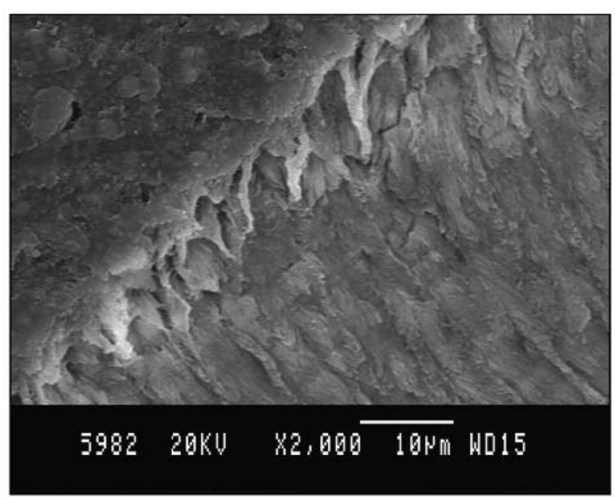

Figure 2. Resin Tags of Conventional Techniques (CST)

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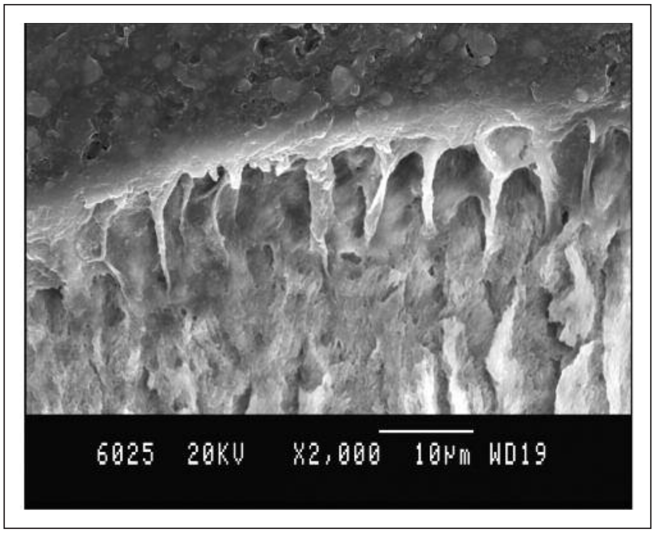

Figure 3. Resin Tags of Enameloplasty Techniques (EST)

which was similar to the results of the present study where, significant microleakage was seen in CST and Fissurotomy technique (FT) with minimal resin tag penetration (Fig. 4).

In the present study, when comparison was done between EST and FT technique, the later showed less resin tag penetration and more microleakage. The probable reason may be the lesser surface area that is provided by the preparation in FT and also the viscosity of the flowable resin did not allow significant penetration.

Droz *et al* evaluated the sealant penetration of a resin composite system, a compomer system and resin modified GIC in artificially grooved fissures in human molars and using EST and FT techniques and reported wide preparation done in EST is beneficial in resin tag penetration rather than narrow penetration in FT with less resin tag penetration thus affecting adaptation.[23]

Blackwood and others reported that acid etching aids in the resin tag penetration by providing micromechanical retention of the sealant to the enamel interface and served as a primary mechanism by which sealants bonded to enamel fissures as demonstrated in SEM analysis. Also, it has been reported that EST along with acid etching gives excellent resin tag penetration.[6]

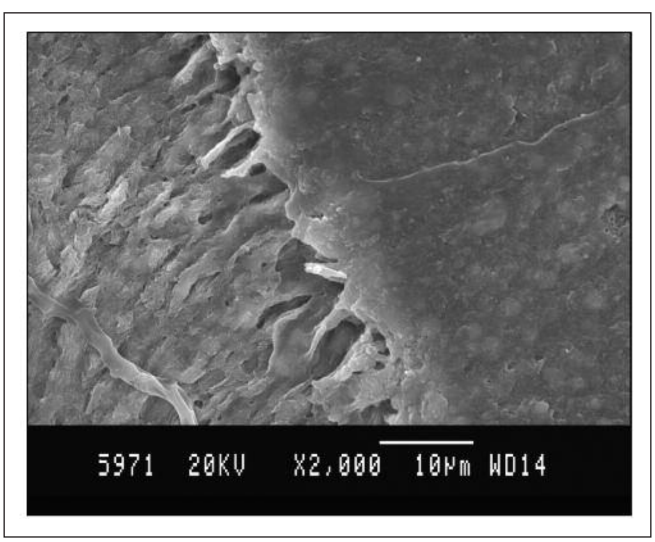

Figure 4. Resin Tags of Fissurotomy Techniques (FT)

The ideal etching time for intact enamel is, however, a controversial point of discussion. Tandon *et al*[24] showed that the sealant bond strength in permanent teeth increased with a longer etching time. Longer etching time of 40 seconds was applied because fissure enamel was considered resistant to etching which was attributable to a ring of aprismatic enamel around fissure entrance and walls. Aprismatic enamel crystals exhibit a unidirectional orientation and are densely arranged. Furthermore, it was suggested that a longer etching time may permit the etchant to penetrate into deep fissures, condition more enamel surfaces and account for the enhanced resin adaptation.[25]

In the present study it was observed that, significant correlation existed between microleakage and resin tag penetration of flowable composite. Lesser microleakage and greater resin tag penetration was reported in EST when compared to CST and FT, where more microleakage and lesser resin tag penetration was observed which was similar to the findings of Duangthip.[26] Hannig *et al* also reported a correlation with microleakage and SEM observation, which supported the findings obtained in the present study.[27] It was regarded that EST provided more surface area to retain the sealant and a thicker layer of sealant was obtained rather than the thin layer resulting after CST.[22]

Kwon reported contrasting results, where the patterns of resin tag penetration of the 4 sealant materials were compared using SEM and showed a low correlation between the formation of resin tags and microleakage, even though the formation of the resin tags is one of the indicators for predicting the sealing ability.[21]

Another reason for the better adaptation and retention of the sealants placed on the enlarged pits and fissures could be that, bur treatment removes the outermost layer of prismless enamel could enhance the deeper penetration of resin leading to the success of EST.[28,29] Gold substantiated that mechanical preparation provides better access to deeper fissures thus, enabling removal of debris and deeper resin penetration.[7]

However, this *in vitro* study needs further *in vivo* studies, because this laboratory test was done using extracted teeth without regarding the circumstances of the oral cavity where isolation is of prime importance for sealant adaptation and under realistic physiological conditions, which may adversely affect dentin bonding and sealant adaptation. Mechanical stresses seems to significantly affect the marginal sealing, could have been assessed. Wear/Abrasion resistance of the flowable composite as a sealant should be tested in further studies before the flowable resin is extensively used as a sealant.

CONCLUSION

EST showed significantly less microleakage and longer resin tag penetration proving to be a reliable preventive method for fissure preparation.

There was no significant difference between CST and FT.

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