Effect of Tooth Preparation on Sealant Success—An *in vitro* Study

Priya Subramaniam * / KL Girish Babu ** / HK Naveen ***

This study compared the depth of penetration and marginal leakage of filled and unfilled sealants, with and without tooth preparation. One hundred and twenty extracted human third molar teeth that were free of restorations, fluorosis, caries, and sealants were used. After adequate storage and surface debridement, the teeth were randomly divided into four groups of 30 teeth each. In two groups, the occlusal surfaces were left intact, while in the other two groups, the occlusal surfaces were prepared using a diamond bur. Teeth in two groups were sealed with a filled sealant, and an unfilled sealant was used to seal teeth in the other two groups. The sealed teeth were then prepared for marginal leakage, immersed in 5% methylene blue (at 37ºC) for 24 hours. Subsequently, buccolingual sections were made and each section was examined for sealant penetration and marginal leakage, using a stereomicroscope. The depth of dye penetration and marginal leakage was evaluated according to a method described by Ovrebo and Raadal. The unfilled sealant placed after tooth preparation showed better enamel fissure penetration and less marginal leakage than the filled sealant.

Keywords: *Pit and fissure sealants; marginal leakage; tooth preparation.*
J Clin Pediatr Dent 33(4): 325–332, 2009

INTRODUCTION

Pits and fissures are generally considered faults or imperfections in cuspal odontogenesis. They have been considered as the single most important feature leading to development of occlusal caries.[1] The complex morphology of occlusal pits and fissures makes them an ideal site for retention of bacteria and food remnants, rendering the performance of proper hygiene difficult or even impossible.[2]

A pit and fissure sealant is a resin material that is introduced into the pits and fissures of caries-susceptible teeth, forming a micromechanically retained physically protective layer that acts to prevent demineralization of enamel by blocking the interaction of cariogenic bacteria and their nutrient substrates, thus eliminating the harmful acidic by-products.[3] The properties required of an ideal fissure sealant include biocompatibility, anticariogenicity, adequate bond strength, good marginal integrity, resistance to abrasion and wear, and cost effectiveness.[4]

The clinical efficacy of fissure sealants is directly related to their retention.[5] Retention depends on morphology of pits and fissures, adequate isolation, conditioning of enamel, application techniques, particular material characteristics like viscosity or surface tension, and adequate adhesion (i.e. penetration of the material into the previously etched system of fissures).[6] Penetration in turn depends on the geometric configuration of the fissures, deposition of sealant material in the latter, physio-chemical characteristics of the sealant, and polymerization shrinkage of the sealant.

The different methods recommended to improve sealant retention include cleaning of the occlusal surface prior to sealant placement with hydrogen peroxide, pumice prophylaxis, air polishing, mechanical preparation of fissures and air abrasion. Preparation of fissures with burs has been suggested to provide better access to the deeper areas of the fissures, thus enabling debris removal and deeper sealant penetration.[7]

Another important factor for sealant success is its marginal integrity, which can be appreciated by evaluating microleakage. Microleakage or marginal leakage may be defined as the ingress of oral fluids into the space between the tooth and restorative material.[8] Microleakage may

* Priya Subramaniam, MDS, Professor and Head, Department of Pedodontics and Preventive Dentistry. The Oxford Dental College, Hospital and Research Center, Bommanahalli, Hosur Road, Bangalore
** KL Girish Babu, MDS, Senior Lecturer, Department of Pedodontics and Preventive Dentistry, The Oxford Dental College, Hospital and Research Center, Bommanahalli, Hosur Road, Bangalore
*** HK Naveen, MDS Former Post Graduate Student, Department of Pedodontics and Preventive Dentistry. The Oxford Dental College, Hospital and Research Center, Bommanahalli, Hosur Road, Bangalore

Send all correspondence to: Dr. Priya Subramaniam, Department of Pedodontics and Preventive Dentistry, The Oxford Dental College, Hospital And Research Centre, Bommanahalli, Hosur Road, Bangalore-560068, Karnataka, India.

Telephone No: + 91 9844225624
Fax No: + 91 08025734656

E -Mail: drpriyapedo@yahoo.com

support the caries process beneath the sealant, so the ability of the sealant to adequately seal the pit or fissure and prevent microleakage is important.

Sealant efficiency can be evaluated *in vivo* or *in vitro*. *In vitro* studies make it possible to assess marginal leakage and predict the marginal sealing capacity of the different materials used for fissure sealing. Important parameters which influence retention and longevity of the fissure sealing material include marginal leakage, marginal gap, voids and filling defects, and enamel fissure penetration.[9]

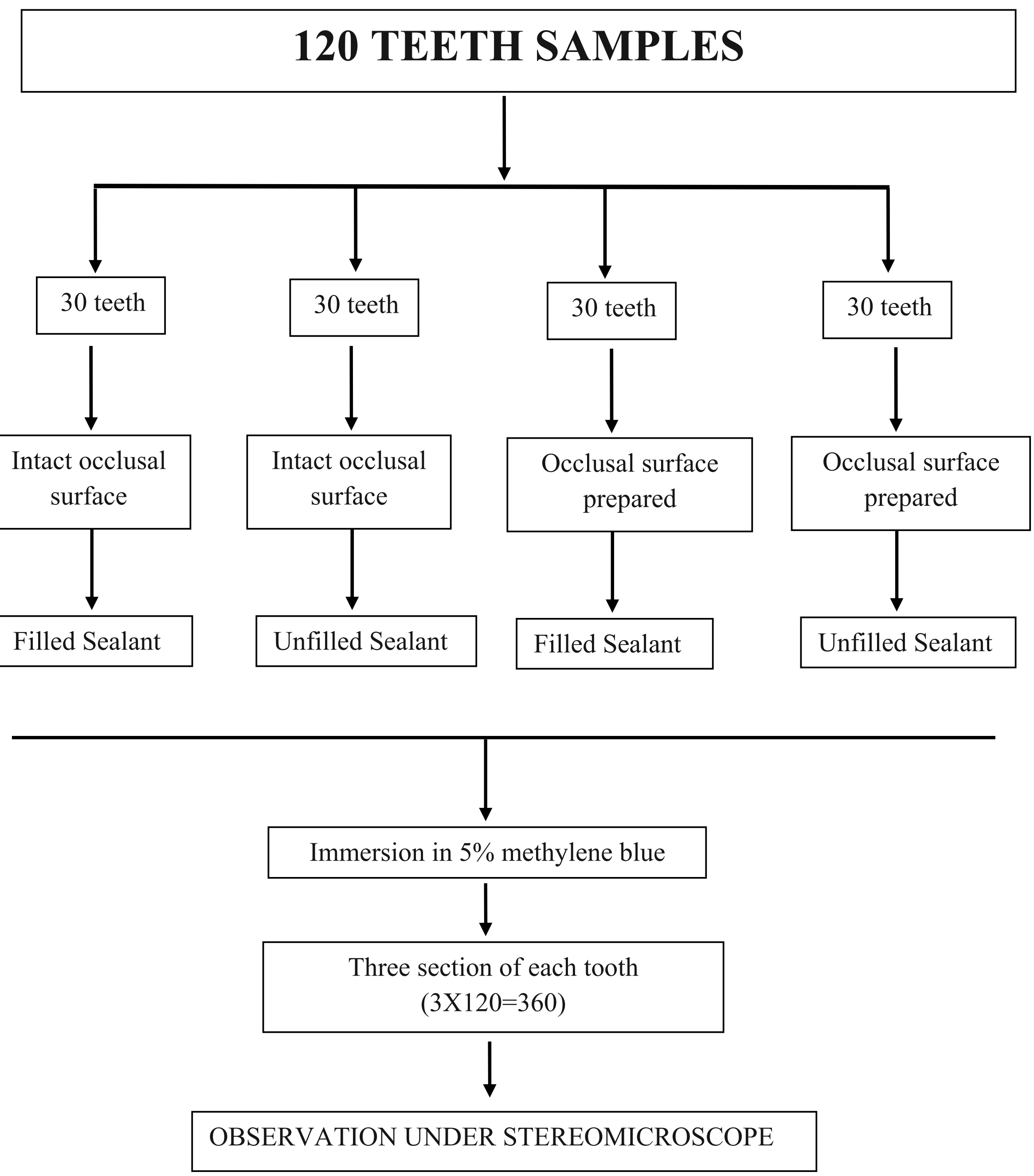
Hence, this study compared the depth of penetration and microleakage of an unfilled sealant, Clinpro (3M, ESPE), with that of a filled sealant, Helioseal-F (Ivoclar, Vivadent); with and without tooth preparation.

MATERIALS AND METHOD

One hundred and twenty freshly extracted sound human permanent third molar teeth, which were collected and stored in 0.1% thymol, were used in this study. The teeth had to be free of restorations, fluorosis, caries and sealants. Hypoplastic permanent third molars, maxillary molars with small occlusal surfaces, molars with incomplete root formation and questionable dental caries were not included.

After surface debridement with hand scaling instruments

Chart 1. Procedure protocol for observation of depth of penetration and marginal microleakage under stereomicroscope



and cleansing with a slurry of pumice, the teeth were randomly divided into four groups, with 30 teeth in each group. In 2 groups, the occlusal surfaces were left intact, while in other 2 groups, the occlusal surfaces were widened (0.5mm) with a tapering fissure diamond bur [FO-21 (MANI Inc, Japan)]. The occlusal surfaces of all teeth were flushed with water for 15 seconds and dried with oil-free compressed air. The surfaces were etched for 15 seconds with 35% phosphoric acid gel (GLUMA etch, Heraeus Kulzar, Germany). The etchant was worked into the intact and prepared fissures with a dental explorer. They were then rinsed with a water spray for 15 seconds before being dried. Both the filled and unfilled resin sealants were applied to teeth with intact occlusal surfaces, as well as to teeth with prepared occlusal surfaces. Both the sealants were applied according to manufacturer's instructions. Care was taken not to incorporate air bubbles. If present, they were removed with an explorer. The sealants were light cured for 20 seconds using visible light cure unit (Selector L.A.500, Taiwan. R.O.C. with a wavelength of 450-490 nanometers). The resin sealant which was initially pink on application turned light yellow after polymerization.

The treated teeth were then stored in sealed containers containing distilled water in a laboratory oven (Labomed, USA) at 37ºC for 24 hours. Subsequently, the apices of all the teeth were sealed with autopolymerising acrylic resin. All tooth surfaces were triple coated with finger nail varnish, with the exception of a 0.5-1.0 mm window around the sealant margins. The teeth were immersed in 5% methylene blue for 24 hours, after which they were rinsed in tap water and the superficial dye was gently removed with pumice slurry and rubber cup. Each tooth was embedded upto to the cementoenamel junction in autopolymerizing acrylic resin. A diamond disc at slow speed was used to section the teeth longitudinally in a bucco-lingual direction. Three sections, each of approximately 2mm thick were obtained for every tooth. The 360 sections of all four treatment groups (90 sections per group) were examined under a stereomicroscope (Lawrence and Mayo, USA) at 32 X magnification (Chart 1). Ordinal rating scores were given for marginal dye penetration, marginal gap, voids in the sealant and completeness of penetration of the sealant into the fissures, as given by Ovrebo and Raadal.[10] The scoring of each parameter for all 120 teeth was recorded on a data sheet. The data obtained was tabulated and subjected to statistical analysis using Kruskal-Wallis one way ANOVA and Tukey type multiple range tests.

RESULTS

The filled sealant, in teeth without preparation, showed a mean value of 4.93 and the mean value for marginal gap was 2.37. Voids and filling defects showed a mean value of 2.37 and for enamel fissure penetration, the mean value was 2.60. Whereas the marginal leakage of the unfilled sealant in teeth without preparation showed a mean value of 4.53. The mean value for marginal gap was 1.97 and for voids and filling defects it was 2.07. For enamel fissure penetration, mean value was 2.13. No significant differences were observed between filled and unfilled sealants, without tooth preparation for marginal leakage, marginal gap and for voids and filling defects. Enamel fissure penetration was higher in the teeth sealed with the unfilled sealant and this was statistically significant (Table 1, Graph 1).

With the filled sealant on prepared occlusal surfaces, the mean values for marginal leakage and marginal gap were 4.23 and 1.77, respectively. Voids and filling defects showed a mean value of 1.60 and for enamel fissure penetration it was 2.17. The unfilled sealant on prepared occlusal surfaces showed mean values of 2.87 for marginal leakage and 1.47 for marginal gap. The mean value for voids and filling defects was 1.53 and for enamel fissure penetration, it was 1.90. There were no significant differences between the filled and unfilled sealants, on teeth with prepared occlusal surfaces for all parameters, except for marginal leakage, which was significantly lesser in teeth sealed with the unfilled sealant (Table 2, Graph 2).

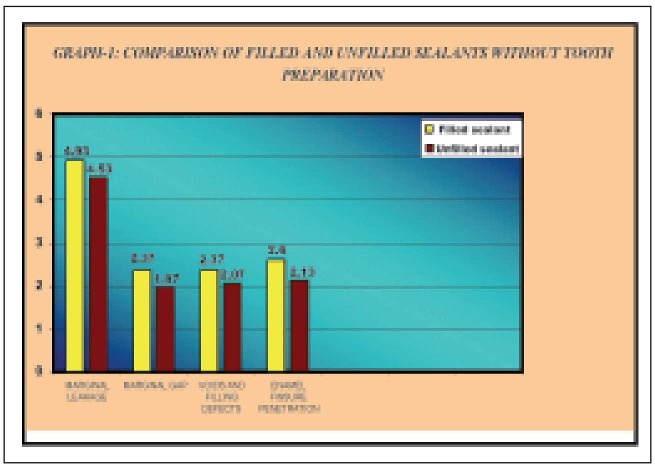
With the filled sealant, there were no significant differences in the marginal leakage, voids and filling defects and enamel fissure penetration, with and without tooth preparation. However, marginal gap was significantly less in the teeth with prepared occlusal surfaces, compared to the teeth with unprepared occlusal surfaces.

Similarly, no significant difference was found in the marginal gap, voids and filling defects and enamel fissure

Table 1. Comparison of filled and unfilled sealants without tooth preparation

Groups	Marginal leakage Mean ± SD	Marginal gap Mean ± SD	Voids & Filling Defects Mean ± SD	Enamel fissure penetration Mean ± SD
Filled Sealant	4.93 ± 1.26	2.37 ± 0.77	2.37 ± 1.27	2.60 ± 0.62
Unfilled Sealant	4.53 ± 2.74	1.97 ± 0.72	2.07 ± 1.19	2.13 ± 1.01
P value	0.812	0.293	0.797	0.166 *

*Significant

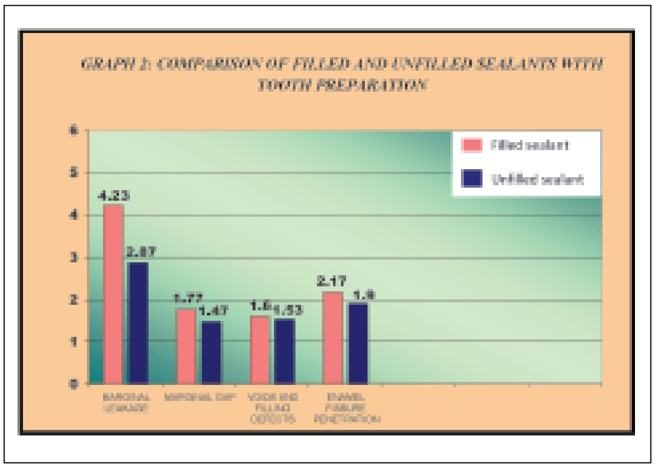


Graph 1. Comparison of filled and unfilled sealants without tooth preparation

Table 2. Comparison of filled and unfilled sealants with tooth preparation

Groups	Marginal leakage Mean ± SD	Marginal gap Mean ± SD	Voids & Filling Defects Mean ± SD	Enamel fissure penetration Mean ± SD
Filled Sealant	4.23 ± 2.70	1.77 ± 0.97	1.60 ± 1.07	2.17 ± 0.91
Unfilled Sealant	2.87 ± 1.57	1.47 ± 1.01	1.53 ± 1.07	1.90 ± 0.89
P value	0.076 *	0.547	0.997	0.635

* - Significant

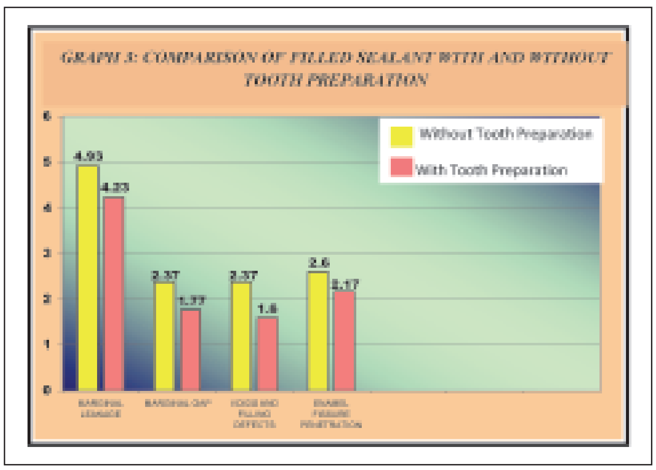


Graph 2. Comparison of filled and unfilled sealants with tooth preparation

Table 3. Comparison of filled sealant with and without tooth preparation

Filled sealant	Marginal leakage Mean ± SD	Marginal gap Mean ± SD	Voids & Filling Defects Mean ± SD	Enamel fissure penetration Mean ± SD
Without Tooth Preparation	4.93 ± 1.26	2.37 ± 0.77	2.37 ± 1.27	2.60 ± 0.62
With Tooth Preparation	4.23 ± 2.70	1.77 ± 0.97	1.60 ± 1.07	2.17 ± 0.91
P value	0.598	0.004 *	0.096	0.220

* - Significant

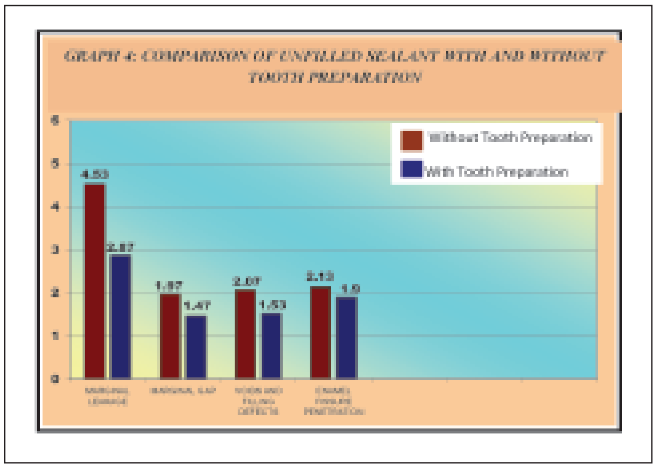


Graph 3. Comparison of filled sealant with and without tooth preparaton

Table 4. Comparison of unfilled sealant with and without tooth preparation

Unfilled sealant	Marginal leakage Mean ± SD	Marginal gap Mean ± SD	Voids & Filling Defects Mean ± SD	Enamel fissure penetration Mean ± SD
Without Tooth Preparation	4.53 ± 2.74	1.97 ± 0.72	2.07 ± 1.19	2.13 ± 1.01
With Tooth Preparation	2.87 ± 1.57	1.47 ± 1.01	1.53 ± 1.07	1.90 ± 0.89
P value	0.019 *	0.812	0.369	0.726

* - Significant



Graph 4. Comparison of unfilled sealant with and without tooth preparation

penetration of the unfilled sealant either with or without tooth preparation. However, marginal leakage was significantly lesser in teeth with prepared occlusal surfaces (Tables 3 and 4, Graphs 3 and 4).

DISCUSSION

The rationale of using pit and fissure sealants is that, when applied on the caries prone fissures, it penetrates these pits and fissures and seals them from the oral environment. A number of local factors influence sealant penetration into pits and fissures irrespective of the nature and type of sealant. Salivary pellicle, organic debris, and handpiece lubricating oil have all been identified as possible contaminants of the tooth surface.[11] The presence of such contaminants blocks the natural porosity of enamel and sealant union, which makes it necessary to do a prophylaxis before sealant application. This is also important for inhibition of marginal leakage. Sealing efficacy is determined by the pretreatment of enamel, that is, prophylaxis, etching, washing and drying and its effects on enamel surface energy.

The use of pumice slurry at slow speed handpiece to clean the tooth surface is the method most widely accepted. However, most studies[12,13] have shown that pumice prophylaxis does not completely and consistently remove the pellicle and dentin, especially in the depth of the fissure. Deposits on the enamel walls within deeper portions of the

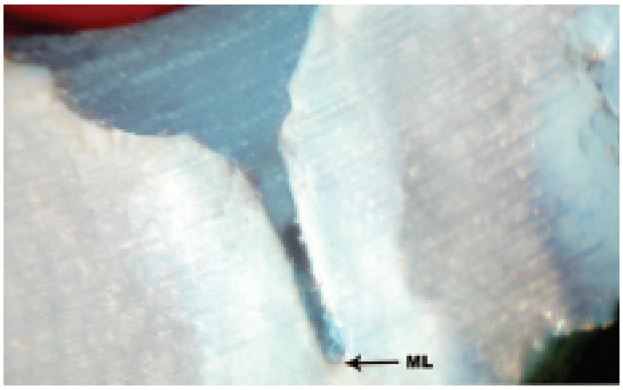


Figure 1. Marginal Leakage (ML) seen with a filled resin sealant without tooth preparation.

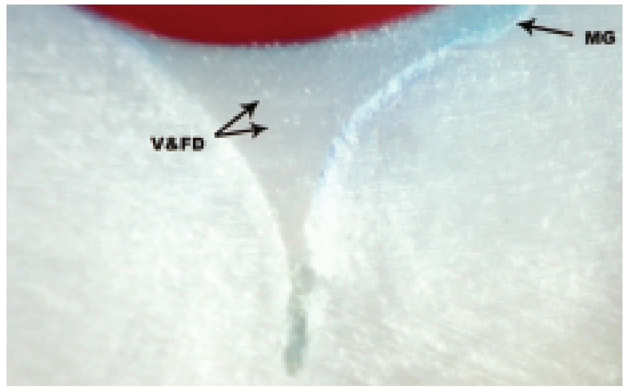


Figure 2. Marginal Gap (MG) and Voids and Filled Defects (V & FD) seen with unfilled resin sealant without tooth preparation.

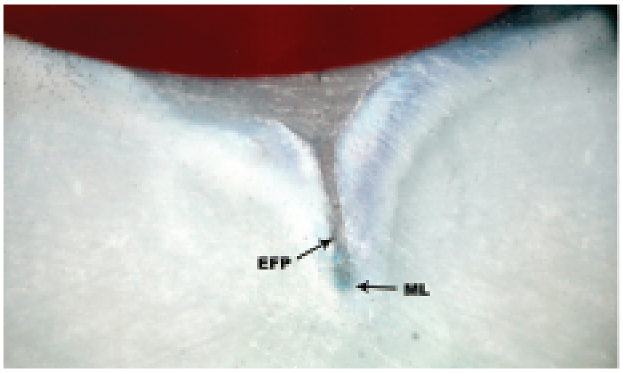


Figure 3. Enamel Fissure Penetration (EFP) & Marginal Leakage (ML) seen with filled resin sealant with tooth preparation.

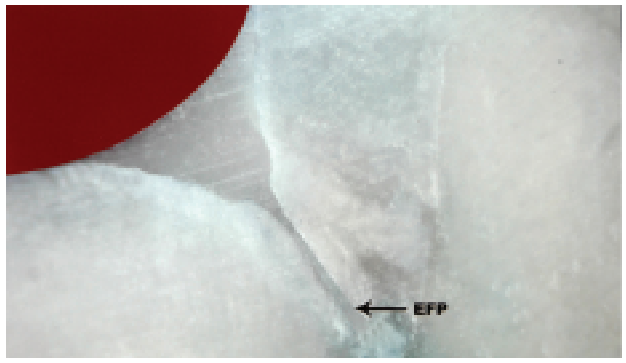


Figure 4. Enamel Fissure Penetration (EFP) seen with unfilled resin sealant with tooth preparation.

fissures serve as a barrier to the action of the acid etching agent.

Alternative methods, such as bur preparation and air abrasion have been proposed to clean pits and fissures of debris. An invasive technique for preparation of the occlusal surface was researched by De Craene and Colls and it consisted of a clinical procedure permitting to establish the presence of caries on occlusal surface through a preventive opening with minimal removal of dentinal tissues.[14]

For mechanical preparation different types of burs have been used.[7, 8, 9,15, 16, 17] In an *in vitro* study, Gieger *et al* found considerably less marginal leakage with a tapered fissure diamond bur than that of a round carbide bur.[7] This could be due to smoothening of fissure walls and easier removal of debris by the tapered diamond bur, which would then increase sealant adhesion to enamel. Since sealants bond to cuspal inclined planes and not to the bottom of pits and fissures, enameloplasty would be necessary for improved adaptation. Enameloplasty technique is specially indicated for deep narrow discoloured fissures, suspected of being carious. Opening of the fissure promotes mechanical retention, reduces micro-leakage and most important, it permits diagnosis of the presence or extent of the carious lesion.[18] From the microbiological aspect, it is the most rational of the invasive methods.[9] Bur treatment removes the outermost layer of prismless enamel seen not only in primary and newly erupted permanent teeth, but also in the fissure walls.[19] It also provides the cleanest surface.[20] The increased surface area obtained with enameloplasty allows for a thicker layer of sealant to be applied, which would be more wear resistant.

Enamel or fissure enlargement with a bur enhances retention by allowing deeper penetration of etchant and sealant, increases surface area for bonding and results in superior sealant adaptation.[21,22] In this study, both filled and unfilled sealants exhibited better sealant penetration, when the occlusal surfaces were prepared.

With regard to marginal leakage, there is a dichotomy of results. Various studies have shown that bur preparation followed with acid etching produces sealants with less marginal leakage than conventional methods.[9,7,8,15] This was in accordance with our study, where we observed less marginal leakage with sealants placed after tooth preparation, as compared to the teeth treated only with pumice prophylaxis and acid etching before sealant placement. The difference in marginal leakage between the prepared and unprepared tooth surfaces could be attributed not only to the depth of penetration of the sealant but could also be due to smoothening of the fissure walls by the diamond bur and the effective removal of debris.

Also, the marginal gap was found to reduce with filled sealants following tooth preparation. However, Eakle *et al* found significantly less marginal leakage in the conventional pumice prophylaxis group. This difference may be due to variation in enamel preparations.[23]

The successful bonding to enamel is dependent on adequate and proper conditioning of enamel. Phosphoric acid has been used in concentrations of 30-50%, with the etching time ranging from 5-120 seconds.[24] With the reduction of etching times, more enamel is preserved without affecting the clinical adhesion of the sealant. On acid etching the enamel surface which is usually in a low energy, weakly reactive, hydrophobic state becomes a high energy, strongly reactive hydrophilic surface.[25] This surface provides a rapid attraction of the sealant. Mechanical retention of sealants is the direct result of resin penetration into this porous etched enamel, forming resinous tags. O' Brien *et al* suggested that when a fissure sealant is painted on the occlusal surface, trapped air in the fissure prevents further penetration after an equilibrium position is reached, resulting in improper penetration.[12]

Resin sealants which posses both low viscosity and excellent wetting properties have been recommended for dental use.[26] For a liquid to flow over a solid, the surface energy of the solid must be greater than the surface tension of the liquid. With the low viscous sealant there is greater potential for the sealant to flow, spread more rapidly over the surface and penetrate. Allowing a sealant to penetrate as long as possible prior to polymerization is also important to obtain satisfactory sealant penetration.

Addition of filler particles, in order to increase the wear resistance of sealants, lowers their ability to penetrate into fissures and micro-porosities of etched enamel. At times the size of the filler particles may be larger than the porosities of the enamel. Faster penetration rates are found with larger holes, denser liquids and those with high surface tension. In our study also, enamel fissure penetration was observed to be superior with the unfilled sealant with tooth preparation. It was also seen that penetration of the filled sealant into the fissures improved considerably following tooth preparation.

Marginal leakage assessment may be qualitative or quantitative with different systems including both simple and computer based methods. Dye penetration has been used in several studies,[8, 27] to assess the presence of marginal leakage around the sealant-enamel surface. The use of different testing substances or dyes makes comparison between studies difficult. Dyes such as methylene blue, basic fuschin and silver nitrate that have small molecules have been used.[8]

Unfilled sealants showed less of marginal gap compared to filled sealants. Also, voids and filling defects were seen to be significantly more in filled sealants without tooth preparation. One of the reasons for marginal gap formation could be the weakening of adhesive bonds by dimensional changes that occur when the materials set. It has been shown that the sealant in a prepared or overfilled fissure may undergo significant shrinkage (approximately 1.5-4%) during curing.[7] The shrinkage takes place either at the pre or post gel process.[7,9] Specifically, for a sealant-covered surface area with a diameter of 4-5 mm; a linear shrinkage of 60-200 micrometers can be expected. The displacement may be large compared to the elasticity of sealant and enamel, leading to splitting at the interface and marginal failure. This may enhance detachment from the surface and result in gap formation.[7] In such situations, inspite of sealant penetration there is leakage of the dye into the fissures at the sealant-enamel interface. In sealants filled exactly to the border of the fissure, the dimensions are significantly lower, resulting in much lower linear displacements.

The presence of a dye in the under-penetrated zone of etched enamel can indicate a susceptible marginal leakage pathway. Clinically, this may imply that the remaining etched area could be a factor for development of caries by microleakage, if the sealant was partially or completely lost.

CONCLUSION

1. The depth of penetration of the unfilled resin sealant was found to be superior to that of the filled resin sealant.
2. Marginal leakage of the filled resin sealant was found to be significantly more compared to that of the unfilled resin sealant.
3. Depth of penetration was more, and marginal leakage was significantly less in both unfilled and filled resin sealants, when applied after tooth preparation.
4. The unfilled resin sealant was superior to the filled resin sealant.

Therefore preparation of the occlusal surfaces prior to sealant application can reduce the chances of marginal leakage as well as increase the depth of penetration of the sealants into occlusal pits and fissures.

REFERENCES

1. Taylor C.L. and Gwinnett A.J. A study of the penetration of sealants into pits and fissures. J Am Dent Assoc, 87: 1181–1188, 1973.
2. Hicks M.J., Flaitz C.M, Garcio-Godoy F. Fluoride releasing sealants and caries like enamel lesion formation in vitro. J Clin Pediatr Dent, 24: 215–219, 2000.
3. Braham RL, Morris ME. Textbook of Pediatric Dentistry. Second edition. CB Publishers and Distributors; 1990.
4. Perez-Lajarin L, Cortes-Lillo O, Garcia-Ballesta C, Cozar-Hidalgo A. Marginal Microleakage of Two Fissure Sealants: A Comparative Study. J Dent Child, 70: 24–28, 2003.
5. Waggoner WF & Siegal M. Pit and fissures Sealant application: Updating the technique. J Am Dent Assoc, 127: 351–361, 1996.
6. Droz D, Schilee MJ, Panight MM. Penetration and Microleakage of Dental Sealants in Artificial Fissures. J Dent Child, 71: 31–44, 2004.
7. Geiger SB, Gulayev S, Weiss EL. Improving fissure sealant quality; mechanical preparation and filling level. J Dent, 28: 407–412, 2000.
8. Hatibovic-Kofman S, Wright GZ, Braverman I. Microleakage of sealants after conventional, bur, and air-abrasion preparation of pits and fissures. Pediatr Dent, 20: 173–176, 1998.
9. Salama FS, Al-Hammad NS. Marginal seal of sealant and compomer materials with and without enameloplasty. Int J Pediatr Dent, 20, 28–32, 2002.
10. Overbo RC, Raadal M. 1990 "Microleakage in fissures sealed with resin or glass Ionomer cement. Scand J Dent Res, 98: 66–69, 1990.

11. Blackwood JA, Dilley DC, Roberts MW, Jr Swift EJ. Evaluation of pumice, fissure enameloplasty and air abrasion on sealant microleakage. Pediatr Dent, 24: 199–203, 2002.
12. Garcio-Godoy F, Gwinett AJ. An SEM study of fissure surfaces conditioned with scraping technique. Clin Prev Dent, 9: 9–13, 1987.
13. Duangthip D, Lussi D. Effects of Fissure Cleaning Methods, Drying Agents, Fissure Morphology on Microleakage and Penetration Ability of Sealants– *in vitro*. Pediatr Dent, 25: 527–532, 2003.
14. De Craene GP, Martens C, Dermaut R. The invasive pit-and-fissure sealing technique in pediatric dentistry: an SEM study of preventive restoration. J Dent Child, 55: 34–42, 1988.
15. Garcia-Godoy F, de Araujo FB. Enhancement of fissure sealant penetration and adaptation: The Enameloplasty technique. J Clin Pediatr Dent, 19: 13–18, 1994.
16. Feldens EG, Feldens CA, de Araujo FB, Souza MAL. Invasive technique of pit and fissure sealants in primary molars: A SEM study. J Clin Pediatr Dent, 18: 187–190, 1994.
17. Theodoridou-Pahini, Tolidis K, Papadogiannis Y. Degree of microleakage of some Pit and fissure sealants: an in vitro study. Int J Pediatr Dent, 6: 173–176, 1996.
18. Shapira JE. Six-year clinical evaluation of fissure sealants placed after mechanical Preparation: a matched pair study. Pediatr Dent, 8: 204–205, 1986.
19. Burrow MP, Burrow JF, Makinson OF. Pits and fissures: etch resistance in prismless enamel walls. Aust Dent J, 46; 258–262, 2001.
20. Jr Pope BD, Garcio-Godoy F, Summitt JB, Chan DDCN. Effectiveness of occlusal fissure cleansing methods and sealant micromorphology. J Dent Child, 175–180, 1996.
21. Xalabarde A, Garcio-Godoy F, Boj JR, Canaida C. Fissure micromorphology and sealant adaptation after occlusal enameloplasty. J Clin Pediatr Dent, 20: 299–304, 1996.
22. Marchushamer M, Neuman E, Garcio-Godoy F. Fluoridated and non-fluoridated unfilled sealants show similar shear strength. Pediatr Dent, 19: 289–290, 1997.
23. Eakle WS, Wong J, Huang H. Microleakage with microabrasion versus acid etched enamel and dentin. J Dent Res, 74: 31, 1995.
24. Brown JR & Barkmeier WW. A comparison of six enamel treatment procedures for sealant bonding. Pediatr Dent, 18: 29–31, 1996.
25. Mathewson RJ and Primosh RE. Fundamentals of Pediatric Dentistry. 3rd Edition. Quintessence Publishing Co, Inc, 1994.
26. Xalabarde A, Garcio-Godoy F, Boj JR, Canaida C. Fissure micromorphology and sealant adaptation after occlusal enameloplasty. J Clin Pediatr Dent, 20: 299–304, 1996.
27. Fuks AB, Grajower R, Shapira J. In vitro assessment of marginal leakage of sealants placed in permanent molars with different etching times. J Dent Child, 50: 425–428, 1984.