A Comparative Evaluation of Different Methods of Cleaning and Preparing Occlusal Fissures, Before Placement of Pit and Fissure Sealant: A Stereomicroscopic Study

Abstract:

Aim and objective: The study was aimed to comparatively evaluate the effect of different methods of cleaning and preparation of occlusal surfaces on microleakage of pit and fissure sealant.

Materials and method: Eighty (80) sound extracted premolars served as samples and were divided into four categories according to the method of cleaning and preparation of occlusal surfaces. Prior to sealant placement. The groups were: A Dry Brush, B Pumice Slurry Air Polishing and D Prophylaxis Paste. In each group, subsequent to cleaning of occlusal surfaces with the method assigned to, pit and fissure sealant was applied. The samples were then kept in an incubator, thermocycled and then immersed in methylene blue dye. Each tooth was sectioned longitudinally into two halves and observed under stereomicroscope for microleakage using a criteria given by Cooley et al.

Statistical analysis: Discrete microleakage score of groups is summarized in number (n) and percentage (%).

Result: Comparing the microleakage score of four groups, □^{2} test showed significantly different microleakage score among the groups (χ^2 =33.96, P <0.001).

Conclusion: Microleakage score showed both pumice slurry (Group B) and especially air polishing (Group C) to be significantly more superior than prophylaxis paste (Group D) and dry brush (Group A).

Key-words: Pit & fissure sealants, microleakage in dental sealants, preparation of occlusal fissures.

Introduction:

Despite the conscientiousness towards home as well as in-office mouth cleaning procedures, optimal fluoride concentration in water and dietary modifications, occlusal caries is still unavoidable for many children and adolescents due to the conspicuous morphology of pits and fissures Their anatomy renders thorough debridement difficult as an average tooth brush bristle is too big to penetrate most of the fissures.[1]

In 1955, MG Buonocore highlighted the benefits of phosphoric acid as an enamel etching agent. According to his studies, the bonding between resin and enamel can be achieved through acid etching which also improves the marginal integrity of a resin restorative material. This bonding system eventually led to the making of the pit and fissure sealants.[2] A micro mechanically protective layer is formed after applying pit and fissure sealants using resin restorative materials which act as a barrier between oral fluids and

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bacteria, thus eliminates the harmful acidic by- products and prevents demineralization of enamel. This method is considered as one of the treatment modalities for prevention of caries and the success of sealants is evaluated on their ability to maximize retention and prevent microleakage.[2] Microleakage may reduce the efficiency of a sealant by rendering pathway for the materials at the enamel-sealant interface, which further leads to progression of cariogenic process underneath the sealant.[3] This basically relies on the meticulous manipulation technique and not merely on the physical, chemical or biological acceptance as a material. Another important factor that improves the sealing and lessens the microleakage of a sealant is the method of preparation of the occlusal surfaces prior to acid etching and sealant application. Various methods have been investigated in a number of studies and some of these are preparation with dry pointed bristle brush, rubber cup and pumice slurry, widening of fissures using dental burs and recent advances such as, abrasion of surface enamel with sodium bicarbonate/aluminium oxide particles.[4] Since there is no consensus regarding the best method of preparation prior to sealant placement, more comparative studies in this regards are required. Therefore this in-vitro study was aimed to compare the efficacy of different methods of preparation of occlusal pits and fissures prior to sealant application and the outcome measure was microleakage.

Materials and Methods:

This *in vitro* study was carried out in the Department of Paedodontics and Preventive Dentistry and department of Oral Pathology & Microbiology, Career Post Graduate Institute of Dental Sciences and Hospital (CPGIDSH), Lucknow (U.P). Eighty (80) sound extracted premolars served as samples, the samples were rinsed under a tap water and any periodontal tissue attached was scrapped off with the help of periodontal scalers and then stored in thymol 0.1%. The samples were categorized into four groups A, B, C & D having twenty teeth in each, according to the method of cleaning used for preparation of occlusal pits & fissures prior to placement of pit and fissure sealant.

Group A*: Dry brush*: (*mfd* by Ultradent star brush, India) The occlusal fissures were cleaned with a dry pointed bristle brush using a low speed hand piece for 10 seconds

Group B*: Pumice slurry*: The occlusal fissures were cleaned with a slurry of fine flour of pumice *(mfd* by Neelkanth Health Care pvt Ltd, Jodhpur, India) and distilled water using a rubber cup with a low speed hand piece for 10 seconds.

Group C: *Air polishing*: (Air prophyAir polisher unit *mfd* by 3M, USA).The occlusal pits & fissures were cleaned with the hand piece of the prophy polishing pencil with the nozzle kept close to the tooth surface directing the sodium bicarbonate and water spray for 10 seconds.

Group D*: Prophylaxis paste*: (DPI PROPOL *mfd* by Dental Product of India) The occlusal fissures were cleaned with prophylaxis paste using a rubber cup and a low speed hand piece for 10 seconds. In each group, subsequent to cleaning with the respective method, the samples were rinsed with air water spray of the three-way syringe and dried with compressed air. After application of acid etchant and bonding agent, the pit and fissure sealant (3M Clinpro) was applied on each tooth following manufacturer's instructions and cured with visible light curing gun (WOODPECKER Led Curing Light Unit). The samples were then kept in an incubator for 1 day in water at room temperature after which they underwent thermocycling using a digital thermostat (*mfd* by Yorco **Sales Pvt. Ltd., New Delhi, India**) for 500 times in water baths of 5°C and 55°C with a dwell time of 30 seconds**.** After thermocycling two coats of acid resistant paint (*mfd* by Berger Paints, India Limited) was applied with the help of sable hair paint brush on all the tooth surfaces leaving 1mm diameter surrounding the sealant. Sticky wax was used to seal the apices of roots, after which the teeth were soaked in 1% aqueous solution of methylene blue dye for 24 hours. They were then rinsed with distilled water to remove the excess dye. Each tooth was then sectioned longitudinally into two halves with the help of water cooled diamond disc. In each group, 20 teeth were cut into longitudinal sections and therefore, became 40 halves, so the total number of samples became 40×4 = 160 halves. The sections were then observed for microleakage using a Stereomicroscope at magnification of 10 x. The degree of microleakage was scored by using a criteria given by *Cooley et al* 2012. The gradation is as follows:

Grade 0 No marginal penetration by dye.

Grade 1 Marginal penetration along the enamel-sealant interface.

Grade 2 Dye penetrations to a depth of sealant.

Observations and Result:

The outcome measure of the study was microleakage and the objective was to compare the microleakage score (both qualitatively and quantitatively) among four different groups: Group A Dry brush, Group B Pumice slurry, Group C Air polishing, and Group D Prophylaxis paste.

Qualitative assessment:

The qualitative assessment of microleakage score of four groups (Group A, Group B, Group C and Group D) is summarized in Table 1 and in Figure 1. Based on microleakage score, Group C(Air Polishing) was the most superior followed by Group B(Pumice slurry), Group D(prophylaxis paste) and Group A(Dry brush) the least (Group A < Group D < Group B < Group C) . Comparing the microleakage score (0/1/2) frequency (%) of four groups, χ^2 test showed significantly different microleakage score among the groups (χ^2=33.96, P<0.001).

Microleakage score	Group A Dry Brush n=40 Halves (%)	Group B Pumice Slurry n=40 Halves (%)	Group C Air Polishing n=40 Halves (%)	Group D Prophylaxis Paste n=40 Halves (%)	χ^2 value	*P* value
0	11 (28)	21 (53)	32 (80)	16 (40)	33.96	<0.001
1	8 (20)	12 (30)	3 (8)	14 (35)		
2	21 (53)	7 (18)	5 (13)	10 (25)		

Table No. 1: Distribution of microleakage score of four groups.

Score 0: no marginal penetration by dye, 1: marginal penetration along the enamel sealant interface, and 2: dye penetration to depth of sealant. Microleakage score of four groups were summarized in number (n) and percentage (%) and compared by χ^2 test (χ^2 value).

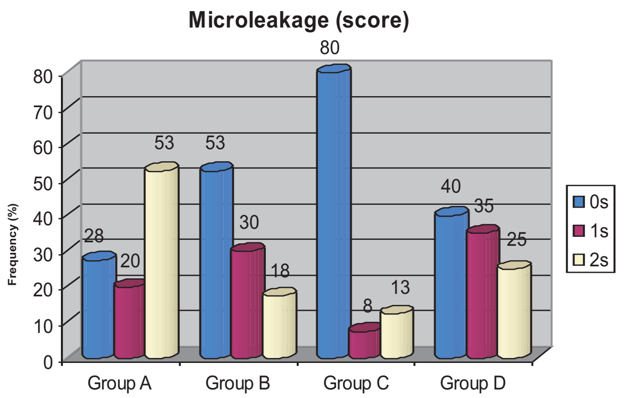


Figure No. 1: Frequency (%) distribution of microleakage score of four groups.

Quantitative assessment:

The quantitative assessment of microleakage score of four groups (Group A, Group B, Group C and Group D) is summarized in Table 2 and Figure 2. The mean microleakage score of Group A (i.e. least superior) was the highest followed by Group D, Group B and Group C (i.e. most superior), the least (Group C < Group B < Group D < Group A).

Comparing the mean microleakage score of four groups, ANOVA showed significantly different microleakage score among the groups (F=9.69, P < 0.001) (Table 3 and Figure 3)

Further, comparing the difference in mean microleakage score between the groups (i.e. inter group) viz. Group A with Group B, Group C and Group D, Tukey test showed significantly (P < 0.01 and P < 0.001, respectively) different and lower microleakage score of both Group B and Group C as compared to Group A but did not differ (P > 0.05) with Group D (Table 3 and Figure. 3).

Similarly, comparing the difference in mean microleakage of Group B with Group C and Group D, Tukey test showed similar (P > 0.05) microleakage score of both Group C and Group D as compared to Group B Similarly, comparing the difference in mean microleakage of Group C with Group D, Tukey test showed significantly (P < 0.05) different and higher microleakage score of Group D as compared to Group C (Table 3)

Group	n	Microleakage score (Mean ± SE)	F value	*P* value
Group A	40 halves	1.25 ± 0.14	9.69	<0.001
Group B	40 halves	0.65 ± 0.12		
Group C	40 halves	0.33 ± 0.11		
Group D	40 halves	0.85 ± 0.13		

Table No. 2: Microleakage score of four groups.

The microleakage score of four groups were summarized in Mean ± SE and compared by ANOVA (F value).

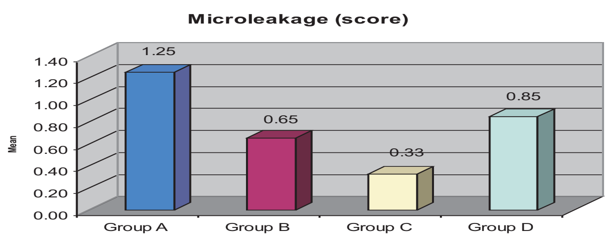
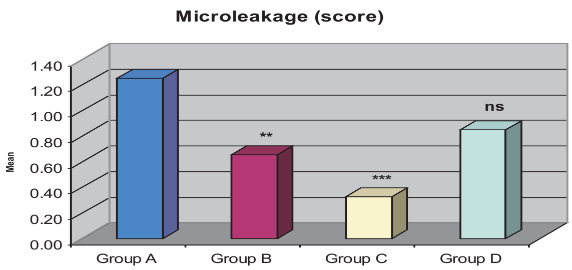


Fig. 2. Mean microleakage score of four groups

Comparison	Mean diff.	q value	*P* value	95% CI of diff.
Group A vs. Group B	0.60	4.83	P < 0.01	0.142 to 1.058
Group A vs. Group C	0.93	7.44	P < 0.001	0.467 to 1.383
Group A vs. Group D	0.40	3.22	P > 0.05	0.058 to 0.858
Group B vs. Group C	0.33	2.61	P > 0.05	0.133 to 0.783
Group B vs. Group D	0.20	1.61	P > 0.05	0.258 to 0.658
Group C vs. Group D	0.53	4.22	P < 0.05	0.067 to 0.983

Table 3: Comparison (*P* value) of difference in mean microleakage score between the groups by Tukey test.

diff: difference, q value: Tukey test value, CI: confidence interval.



$^{ns}P>0.05$ or $^{**}P<0.01$ or $^{***}P<0.001$ - as compared to Group A

Figure. 3: Comparison of difference in mean microleakage score between four groups.

Discussion:

According to the literature the most suitable time for the application of pit & fissure sealants is seen after the eruption of permanent molars because the newly erupted teeth are less mineralized and prone to acid attack. In such a state, early placement of sealants may prevent formation of carious lesions on occlusal pits and fissures. Meticulous application techniques have resulted in high retention rates and increased bond strength of sealants[5]. Contaminants like plaque pellicle inhibit the diffusion of sealant to closely contact the enamel, therefore adequate cleaning and preparation of occlusal surfaces before sealant placement is mandatory to maximize the retention and inhibit the microleakage.[6] Plaque pellicle and other contaminants inhibit the dispersion of sealant and also the ability of sealant to closely contact the enamel. Therefore, proper cleaning of pits and fissure before sealant placement are of the utmost importance, so as to successfully inhibit microleakage and maximize retention.[7]. Sound premolars extracted due to orthodontic reasons were used. As these teeth have remained in the oral cavity for the least amount of time and provide a favorable environment for the application of sealants.[4]

In-vitro studies layout a platform to evaluate and compare the materials prior to the clinical applications without subjecting the humans and animals to their side affects and toxicities therefore should be performed before embarking on to in-vivo studies. Thus the present study was aimed to be an in-vitro one. Thermocycling process exposes dental materials and extracted teeth to the changes in temperature similar to those occurring in the oral cavity. According to *Mohammed Ali Saghri et al (2013)*[8] this was an appropriate artificial aging test. The samples were immersed in a solution of 1% methylene blue dye for 24 hours, similar to a study done by *Birkenfeld et al. (2017).*[9] The molecule size of methylene blue is very small (0.5-0.7nm), even smaller than bacteria; thus, methylene blue 1% solution can penetrate farther than other dyes.[10] Cooley et al *(2012)* criteria was used for the assessment of microleakage score and has been used in several studies viz *Chan D C N et al (1999).*[7] and *Shefali et al (2013)*[11] *Chan DCN et al (1999)[7]* observed penetration of the dye to the base of the sealant in both dry brush and pumice group and were insignificant. *Bogert TR and Garcia-Godoy F (1992)*[12] found an insignificant difference in sealant bond strength on using different prophylaxis methods *viz*: water, pumice, non fluoridated and fluoridated pastes however, non fluoridated paste displayed the highest bond strength as compared to pumice and fluoridated pastes. *James A. et al(1998).[13]* suggested that dry brushing by the operator may be an acceptable alternative to using a rotary instrument with fluoridated paste for the retention of the sealant. *S. Hatibovic-Kofman(1998)*[3] found an insignificant microleakage score between pumice and air abrasion. *Julie A. Blackwood, (2002)*[14] found and insignificant difference in microleakage among enameloplasty, acid etching, air abrasion and pumice methods. *D. Duangthip* et al. *(2003)*[15] concluded that air abrasion did not significantly decrease the microleakage or improve the penetration ability of sealant when compared to traditional pumice prophylaxis. *Frederic Courson et al (2003)*[16] concluded that when teeth are treated with air abrasion or air polishing, sealants have better enamel sealing and penetration ability when compared to pumice. *Anju Singh et al (2020)*[17] found maximum microleakage of pit and fissure sealants with pumice prophylaxis technique as compared to air polishing, fissurotomy, enameloplasty and air abrasion respectively. This was also observed by *Hatibovic-Kofman et al. (1998)*[3], *Chaitra et al (2010)*[18] *and Agarwal and Shigli (2012)*[1] and stated that pumice slurry method is not very effective means of surface preparation. *Rahul J Hedge and Rochelle C Coutinho (2016)*[19] found a higher retention of sealant with pumice slurry and surface conditioning methods as compared to brushing. *Avinash et al (2010)*[20] advocated the use of prophy cup or bristle brush and pumice to clean the surface of pits and fissures. *Agarwal and Shigli (2012)*[1] observed a significantly lesser microleakage.

In In the present study, Air polishing gave the best result by showing the least microleakage score when compared to other methods. The reasons proposed could be: air polishing allows complete removal of extrinsic stains and debris from the base of the fissures as well. It is found to be efficient in many studies.[7] This method bombards abrasive particles of sodium bicarbonate and creates higher surface energy compared to smudging with rubber cup or bristle brush thereby, facilitates the effect of acid etching, resin penetration and results in higher bond strength.[9] Although the mean microleakage score between pumice slurry and air polishing did not differ significantly P >0.05, the microleakage score of pumice slurry was higher than air polishing and was the second best in the study. This difference could be attributed to the fact that pumice slurry does not gets thoroughly removed from pits and fissures even after rinsing thus, prevents enamel conditioning and decreases the resin penetration.[21] Another reason could be that pumice removes only organic material on smooth enamel surfaces and does not adequately cleans the enamel walls of the fissures. The prophy paste showed higher microleakage score than air polishing and pumice slurry. *Comboni S* et al.[22] in a study compared the microscopic observations on human enamel with air polishing and polishing paste. They found complete cleaning of enamel surface down to the tooth microstructures with air polishing, whereas use of polishing paste resulted in enamel surface that appeared abraded, flattened and some of the natural irregular enamel surfaces showed some fillings. These fillings could be the remnants of polishing paste, abraded tooth debris or plaque. Therefore, it may be probable to relate that prophy paste is actually not able to ensure thorough removal of residues than air polishing. These residues could hinder the resin penetration and adaptation of pit and fissure sealants. Among all, the highest microleakage score was observed with dry brush, the possible reason for this could be that cleaning and preparation of the occlusal surfaces with this method is simply confined to the cuspal inclined planes and not to the base of the fissures as the diameter of the bristle is too large (0.2mm) to penetrate the orifice of most fissure measuring 0.1 mm wide as suggested by Newburn (19890[23] Hence, the finding of this study suggest that air polishing and thus may improve the adaptation of pit and fissure sealants, minimize microleakage and likely to participate in caries prevention better than the other methods used. Whether the result of this in-vivo study can be extrapolated to in-vitro conditions is a matter of investigation.

Conclusion:

1. Based on the observations of this in-vitro study all the methods used for preparing the occlusal fissures showed microleakage in pit and fissure sealant. There was a significantly different microleakage score among the techniques used. Air polishing and pumice slurry were found to be significantly superior methods especially air polishing Highest microleakage score was observed in dry brush method followed by prophylaxis paste, pumice slurry and air polishing.
2. To arrive at a definitive conclusion more in-vitro and clinical trials to evaluate the effect of these methods on microleakage in pit and fissure sealants should be conducted with larger sample size and longer follow ups.
3. Pit and fissure sealants may behave in a different manner due to the factors like types of fissures, preparation of fissures, enamel etching and bonding, contamination of prepared surfaces and their physical and chemical properties as well. However, a meticulous technique of application plays an important role that affects their marginal integrity and microleakage.

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