Comparative Evaluation of Penetrative and Adaptive Properties of Unfilled and Filled Resin-Based Sealants When Placed using Conventional acid Etching, Lasing, and Fissurotomy Bur Technique of Enamel Preparation: An *in vitro* Scanning Electron Microscope Study

Abstract

Background: There is a confusion regarding selection of unfilled or filled sealant and method of enamel preparation before sealant application. This study was carried out to compare three techniques of enamel preparation using both unfilled and filled type of sealants. **Objective:** The objective of the study is to assess the penetrative and adaptive ability of filled and unfilled sealants in three techniques of enamel fissure preparations. **Materials and Methods:** Total 36 extracted teeth were divided into 3 groups, each containing 12 samples. The samples of Group A were prepared by conventional acid etching with 37% phosphoric acid, and the Group B was subjected to Er: YAG lasing, while in Group C, fissurotomy followed by acid etching was done. The sealant placement was carried out using split tooth design in all the samples. Assessment of penetration and adaptation was done under scanning electron microscope using the scoring criteria adopted by Kane B *et al*. and Dukic W *et al*. **Results:** Group A and Group C showed better adaptation than Group B. Statistically, no significant difference was observed in the penetration property among three techniques. Similarly, the unfilled and filled sealant showed statistically nonsignificant results for the penetration and adaptation comparison. **Conclusion:** Irrespective of the sealant material selected, the conventional method of acid etching alone or in conjunction with fissurotomy bur for better retentiveness seems to be an acceptable choice of treatment modality. The study will help the clinicians to choose the sealant material and technique of enamel preparation.

Keywords: *Acid etching, adaptation, enamel preparation, fissure sealants, lasing, penetration, pit*

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Submitted : 22-Mar-2021
Revised : 14-Sep-2021
Accepted : 29-Oct-2021
Published : 03-Nov-2022

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Introduction

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.[1]

Penetration of the sealant into the complete depths of pits and fissures, its lateral wall adaptation, and subsequent retention are the key factors in the longevity and thus success of the sealants.[2,3]

For the optimum period of retention of a sealant, its adaptation to the walls of fissures and penetration into the depth of the same form the mainstay of the success of this sealant treatment. Hence, this study was designed to compare both the penetration and adaptation of filled and unfilled pit and fissure sealants in differently prepared occlusal surfaces.

Materials and Methods

After getting the approval of institutional review board and ethics committee, the project was carried out using total 36 teeth with normal morphology, which were collected for the study purpose from the patients in whom extractions were indicated for orthodontic purpose[4] or required removal of being third molars.[5,6] Teeth with caries, restorations in any form, fluorosis, developmental defects, hypoplasia, fractures, cracks,[7,8] and abnormal morphology were excluded from the study.[6]

How to cite this article: Shingare PR, Chaugule V, Pankey N, Kakade P. Comparative evaluation of penetrative and adaptive properties of unfilled and filled resin-based sealants when placed using conventional acid etching, lasing, and fissurotomy bur technique of enamel preparation: An *in vitro* scanning electron microscope study. Contemp Clin Dent 2022;13:349-55.

Access this article online
Website: www.contempclindent.org
DOI: 10.4103/ccd.ccd_227_21
Quick Response Code:

Immediately after extraction, the teeth were cleaned and hand scaled[8,9] after which pumice prophylaxis was done followed by ultrasonic cleaning.[10-12] Detection of caries was carried out with visual examination and sharp explorer.[11,13,14] The teeth were stored in Chloramine T solution at room temperature until further use.[9,11,13]

The total number of samples was further divided into 3 groups, each containing 12 samples and labeled as Group A for conventional acid etching of enamel, Group B treatment of enamel with laser, and Group C treatment of enamel with fissurotomy bur and acid etching.

The occlusal surfaces of all the samples of Group A were cleaned and dried[14] and then etched for 15 s with 37% phosphoric acid gel (LOT 100828-01, Prime Dental Products Pvt. Ltd) according to the manufacturer's instruction.[12] After rinsing and drying,[8,9,14,15] sealant placement was carried out using split tooth design in all the samples. The split tooth design indicates placement of one type of sealant applied on mesial half of the fissure and another sealant applied on distal half of the fissure.[12] Both the sealant materials were applied according to the manufacturer's instructions. Sealrite (Pulpdent Corporation, Watertown, MA 02471-0780 U. S. A. 4.4% filled) was applied to the mesial/distal half of the fissure and allowed to flow for 20 s and light cured for 30 s using dental light cure unit (Hilus). After curing the first sealant (Sealrite), Clinpro (3M ESPE, Dental Products, St. Paul, MN 55144-1000, USA, unfilled) was applied to the remaining mesial/distal half of the fissure in the same manner and cured. The occlusal surfaces of the samples in group B were subjected to Er:YAG laser treatment in noncontact and scanning mode using 350 mJ energy at 6 Hz frequency and a power of 2.1 Watt. A standardized focal distance of 12 mm was maintained with the laser beam directed at right angles to the occlusal surface for 5 s and by keeping unprepared middle portion of 1 mm width with the use of air and water spray.[12,16] Then sealant was applied in the same way as that of for Group A without acid etching. The fissures of the samples from Group C before subjecting to acid etching process were enlarged with carbide fissurotomy bur (Fissurotomy Bur 18010 for molars and 18013 for premolars. SS White, Ivoclar North America, Inc.,) using high-speed airotor handpiece in a light sweeping motion for 10 s[13,17] and by keeping unprepared middle portion of 1 mm width[12] followed by acid etching and sealant application. All the samples were stored in sealed containers containing saline for 1 week at 37°C[4] and then thermocycled at 5°C, 37°C, and 55°C for 500 cycles with a dwell time of 30 s[8,13,18] and again stored for 1 week in saline at 37°C.[4]

Scanning electron microscope examination

Discing was done to obtain longitudinal section in mesiodistal direction[15,19] so that each section contained both the sealants. The sections were polished,[20] cleaned, and fixed[21] followed by sputtering with palladium in JEOL JFC 1600 Auto Fine Coater and subjected to scanning electron microscope (SEM) study for the evaluation of adaptation of sealant to the walls of fissure and for flow and penetration into the depth of fissure at ×40 magnification [Figures 1 and 2, respectively].

Each section was examined and scored independently by two separate examiners qualitatively for penetrative and adaptative ability of individual sealant using the scoring system adapted by Barbara Kane *et al*.[18] and quantitatively for measuring penetration proportion as given by Dukić *et al*.[11]

Scoring for penetration ability

- Score 1 – Sealant penetrated 1/3 of total length of the fissure
- Score 2 – Sealant penetrated 1/2 of total length of the fissure
- Score 3 – Sealant penetrated more than 1/2 of the total length of the fissure but not to the base of the fissure
- Score 4 –Sealant penetrated completely to the base of the fissure.

Scoring for adaptation

- Score 1 – Smooth adaptation. Sealant flows with enamel. No ledges.
- Score 2 – Sealant is not well adapted. Ledge may be present.

Formula to calculate penetration proportion-

$$P = \frac{\text{\% of the depth of sealant penetration}\left(\text{P1}\right)}{\text{Total depth of sealed fissure}\left(\text{P2}\right)}$$

Statistical analysis

Statistical analysis was carried out using the Software Package for Statistical Analysis (SPSS) version 17.0. Chicago: SPSS Inc. The arithmetic means of scores for adaptation, penetration ability, and penetration proportions were calculated in all the groups. Cohen's Kappa coefficient to determine the interexaminer variability was 0.9**.** The comparison among three groups for evaluation of penetrative and adaptative ability using qualitative scores was done by Kruskal–Wallis test at 95% confidence level, while the comparison between two groups was carried out using Mann–Whitney test at 95% confidence level. For comparison of paired observations in the same group, Wilcoxon signed-rank test at 95% confidence level was applied.

Results

The results of the study are summarized in Tables 1-3. Table 1 shows the mean and standard deviation values for penetration ability for Clinpro and Sealrite using

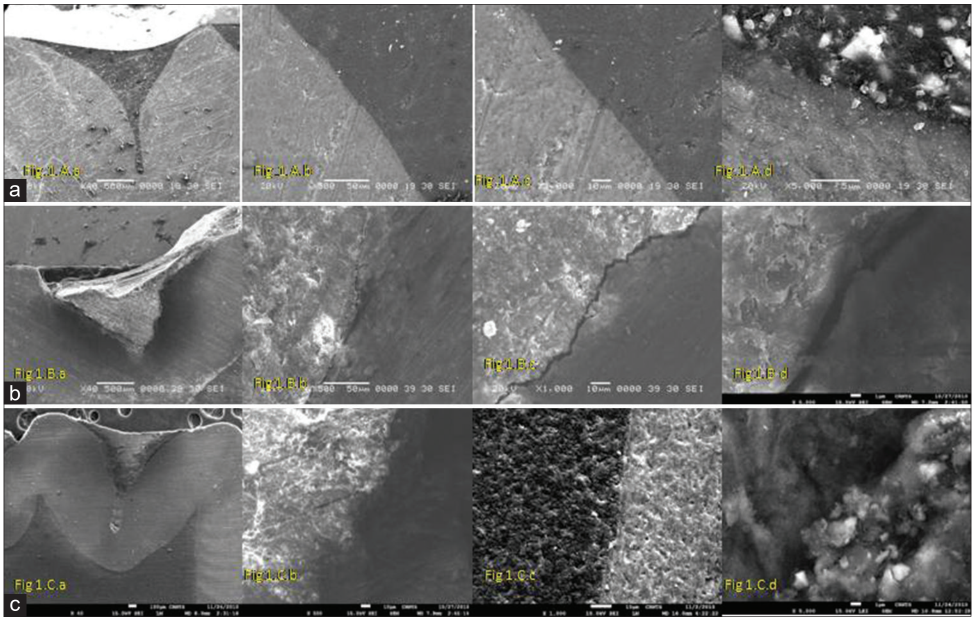

Figure 1: scanning electron microscope images showing adaptation in three techniques of enamel preparation. (a) Sealant applied to enamel in acid etching group at ×40 (1A. a), ×500 (1A. b), ×1000 (1A. c), and ×5000 (1A. d) showing close adaptation of sealant material to the walls of fissure. (b) Sealant applied to enamel in laser group at ×40 (1B. a), ×500 (1B. b), ×1000 (1B. c), and ×5000 (1B. d) showing poor adaptation of sealant material to the walls of fissure. (c) Sealant applied to enamel in fissurotomy group at ×40 (1C. a), ×500 (1C. b), ×1000 (1C. c), and ×5000 (1C. d) showing adaptation of sealant material to the walls of fissure

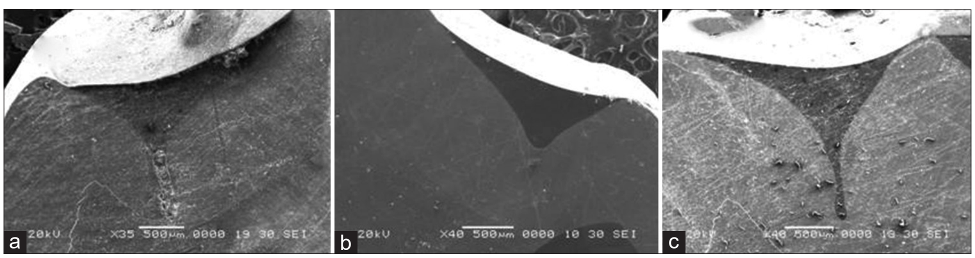

Figure 2: scanning electron microscope image showing penetration ability of sealant at × 40 magnification. (a) Sealant penetrated to half of the length of fissure (b) Sealant penetrated to more than half of the length of fissure (c) Sealant penetrated to the base of the fissure

three techniques of enamel treatment. The Kruskal–Wallis test showed nonsignificant differences in penetration ability scores among the three groups with P value of 0.893 and 0.425 ($P > 0.05$), respectively, for Clinpro and Sealrite. The comparison between two groups was done by Mann–Whitney test which showed nonsignificant differences for all comparisons for Clinpro and Sealrite. None of the sealants showed Score 1 for penetration ability. Table 2 shows the mean and standard deviation values for penetration proportion of Clinpro and Sealrite using three techniques enamel treatment. The analysis of variance test showed nonsignificant differences in penetration proportion among three groups with P value of 0.649 and 0.537 ($P > 0.05$) for Clinpro and Sealrite, respectively. The comparison between two groups of enamel treatment for each material by unpaired *t*-test revealed nonsignificant differences for all comparisons. Table 3 exhibits the comparison of adaptation scores of Clinpro and Sealrite in three groups of enamel treatment. The Kruskal–Wallis test showed significant differences in adaptation among three groups with P value of 0.002 and 0.013 ($P < 0.05$) for Clinpro and Sealrite, respectively. Comparison between two groups of materials was assessed by Mann–Whitney test and it showed significant differences when acid etching and fissurotomy groups were compared with laser group, but when acid etching and fissurotomy group were compared, the difference was statistically nonsignificant. Comparisons of unfilled and filled sealant for each group of enamel treatment showed nonsignificant differences by Wilcoxon signed-rank test for penetration ability and adaptation and by Paired *t*-test for penetration proportion, respectively. Both materials showed nonsignificant differences irrespective of the technique of preparation [Figure 3].

Discussion

Pit and fissure sealants are effective preventive agents as long as they remain bonded to the teeth. Micromechanical adaptation of the sealant is achieved through porosities created by conditioning the enamel conventionally by

Table 1: Comparison of penetration ability for Clinpro and Sealrite sealant among 3 techniques

Group	*n*	Mean	SD	Kruskal–Wallis test χ^2 (df=2)	*P*
Clinpro					
Acid etching (A)	12	3.58	0.515	0.227	0.893 (NS)
Laser (B)	12	3.67	0.492		
Fissurotomy (C)	12	3.58	0.515		
A versus B	12			Mann–Whitney test *Z*: 0.413	0.680 (NS)
A versus C	12			Mann–Whitney test *Z*: 0.0	1.0 (NS)
B versus C	12			Mann–Whitney test *Z*: 0.413	0.680 (NS)
Sealrite					
Acid etching (A)	12	3.50	0.674	1.710	0.425 (NS)
Laser (B)	12	3.33	0.651		
Fissurotomy (C)	12	3.67	0.492		
A versus B	12			Mann–Whitney test *Z*: 0.709	0.479 (NS)
A versus C	12			Mann–Whitney test *Z*: 0.544	0.586 (NS)
B versus C	12			Mann–Whitney test *Z*: 1.316	0.188 (NS)

NS: Nonsignificant, SD: Standard deviation

Table 2: Comparison of penetration proportion for Clinpro and Sealrite sealant among 3 techniques

Sealant	Group	*n*	Mean	SD	ANOVA *F*	*P*
Clinpro	Acid etching (A)	12	0.9378	0.1180	0.438	0.649 (NS)
	Laser (B)	12	0.9691	0.0467		
	Fissurotomy (C)	12	0.9633	0.0816		
	A versus B	12			Unpaired t: 0.854	0.402 (NS)
	A versus C	12			Unpaired t: 0.616	0.544 (NS)
	B versus C	12			Unpaired t: 0.213	0.833 (NS)
Sealrite	Acid etching (A)	12	0.8881	0.1570	0.634	0.537 (NS)
	Laser (B)	12	0.8930	0.1389		
	Fissurotomy (C)	12	0.9481	0.1384		
	A versus B	12			Unpaired t: 0.081	0.936 (NS)
	A versus C	12			Unpaired t: 0.994	0.331 (NS)
	B versus C	12			Unpaired t: 0.974	0.341 (NS)

NS: Nonsignificant, SD: Standard deviation

Table 3: Comparison of adaptation for Clinpro (unfilled) and Sealrite (filled) sealant among 3 techniques

Sealant	Group	*n*	Mean	SD	Kruskal–Wallis test χ^2 (df=2)	*P*
Clinpro	Acid etching (A)	12	1.08	0.289	12.17	0.002 (S)
	Laser (B)	12	1.75	0.452		
	Fissurotomy (C)	12	1.25	0.452		
	A versus B	12			Mann–Whitney test *Z*: 3.24	0.001 (S)
	A versus C	12			Mann–Whitney test *Z*: 1.072	0.284 (NS)
	B versus C	12			Mann–Whitney test *Z*: 2.398	0.016 (S)
Sealrite	Acid etching (A)	12	1.17	0.389	8.667	0.013 (S)
	Laser (B)	12	1.75	0.452		
	Fissurotomy (C)	12	1.33	0.492		
	A versus B	12			Mann–Whitney test *Z*: 2.807	0.005 (S)
	A versus C	12			Mann–Whitney test *Z*: 0.923	0.356 (NS)
	B versus C	12			Mann–Whitney test *Z*: 2.005	0.045 (S)

S: Significant, NS: Nonsignificant, SD: Standard deviation

acid etching, before applying the sealant. Mechanical preparation or enameloplasty involves widening of the fissures using rotary instrumentation to permit better diagnosis of underlying demineralized tissues and increase penetration and surface area for retention of the sealant.

Recent innovative technique is LASER. Preparing the tooth by laser does not require any isolation of tooth and

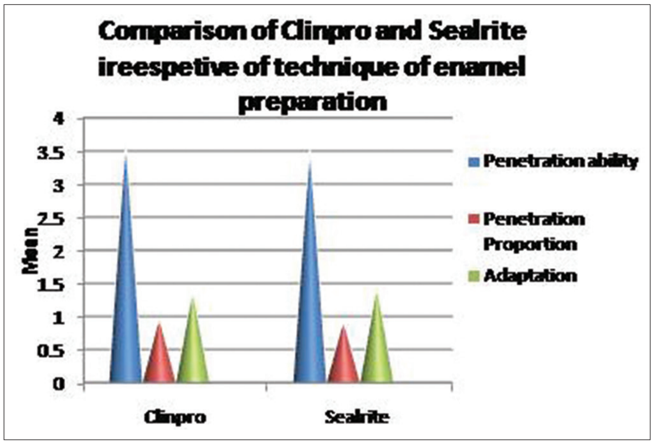

Figure 3: Comparison of Clinpro and Sealrite irrespective of technique of enamel preparation

thus reduce clinical time.[14] There is scarce literature on the quality of pit and fissure sealants placed after surface preparation with laser technique using Er: YAG. As the technique of enamel surface preparation changes, the surface characteristics of enamel are expected to change and the material applied over this may also behave differently with each technique. Therefore, this study was planned to check the behavior of materials (i.e., filled and unfilled) and to find a better combination in terms of material and technique selection.

The results showed that the adaptation of both the materials to the walls of the fissures in acid etching and fissurotomy group was better than that found in the laser group. Examinations done at higher magnification showed complete adaptation of sealants in acid etching and fissurotomy group [Figure 1a and c] whereas the laser-treated samples showed poor adaptation to the walls of fissures and gaps were evident [Figure 1b]. Borsatto *et al*.[22] showed that laser etching did not provide a homogeneous etching of tooth surface and such irregular microstructure led to bonding failures and undermined marginal sealing resulting in more microleakage in laser etching suggesting that laser treatment yielded poor adaptation. Our results too concur with these findings. Selecman *et al*.[10] showed that there was no correlation between microleakage and penetration as shown by simple regression analysis, and total penetration of the sealant material is unnecessary if an adequate bond has occurred coronal to the base of the fissure and adjacent to the cuspal inclines. Hence, the adaptation of sealant to the fissure wall which results in prevention of microleakage is more important for success than the depth of the penetration.

No significant difference was observed in terms of penetration ability between filled and unfilled sealants when applied using three techniques of enamel preparation [Table 1]. which was contrary to the one by Salama and Al-Hammad[9] who showed better penetration in enameloplasty with acid etching. The dissimilar result could be because of the use of different scoring systems in the two studies. We preferred both the qualitative and quantitative scoring criteria for the measurement of penetration ability and penetration proportion.[18,11] Whereas authors[9] had used scoring system utilizing only two scores, namely 0 and 1. The type of burs and method of preparation applied could be another reason. We used fissurotomy carbide bur while the authors used the diamond bur. Widening of the fissures with bur might have created smooth inclined walls thus facilitating the increased resin flow; however, it is an invasive procedure. Our results were also contradictory to those of Durmuglu *et al*.[23] who showed that the significant impacts on penetration ability were due to the fissure type (shape and depth), the material, and the way of application.

Our results were in accordance with those of Memarpour *et al*.[24] and Khogli *et al*.[25] showing no significant differences in sealant penetration when used with bur, laser, and conventional acid etching techniques.

Upon comparing the adaptative and penetrative properties of both the types of sealants, the difference was found to be indistinguishable. Thus, the addition of filler particles to fissure sealant material may seem to have infinitesimal influence on clinical outcomes. However, according to Simonsen, Reddy *et al*. and Naaman *et al*. filled sealants had a higher wear resistance; their ability to penetrate into fissures appeared to be low. The filled sealants usually require occlusal adjustments, which lengthen the procedure unnecessarily. The unfilled resin sealants on the other hand have a lower viscosity and provide easy penetration into fissures and thus heading for a better retention.[26-28]

A lacuna in our study was that the transverse sectioning of the samples could have helped in ascertaining a better micromechanical bonding by observing the penetration of resin tags in the walls of prepared surfaces of fissure enamel.

An important finding to note that the use of LASER involves some difficulties for the operator like deploying the correct angle and tip during preparation, experience, and expertise for the success of laser pretreatment. Some authors believe that laser preparation alone may be as effective as acid etching.[29,30] However, more studies recommended pretreatment with Er: YAG laser combined with acid etching.[31-33] Karaman *et al*. found no significant difference between two enamel preparation methods.[34] Majority of the previous studies demonstrated that the roughened surface produced by the laser alone lacks the seal obtained with acid etching.[35] In contrast, some authors reported that laser irradiation may be used to etch enamel.

The SEM studies of the fissure enamel surface treated with the laser showed a pattern of crater-shaped pits similar to microexplosions corresponding to the laser

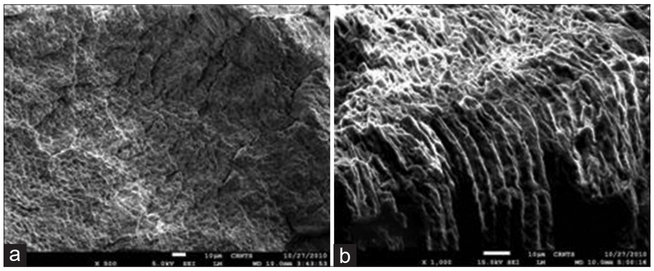

Figure 4: Scanning electron microscope image showing (a) a rough, flaky appearance of the enamel surface after laser treatment (b) Cratering effect with uneven margins, melting with lava-like concrescences after laser treatment of enamel

pulses. This resulted in a rough, flaky appearance of the surface [Figure 4a]. Controlled preparation was very difficult to achieve due to noncontact and discontinuous emission mode. As a result, the pits or microexplosions were not always uniformly distributed on the laser-treated fissure. In some cases, they were present on one fissure wall, with the other wall remaining intact depending upon the incidence of the laser beam. Moreover, the shallow and deeper fissures are likely to be hit only at their entrance and laser beam could not reach the bottom of fissure.[36] This is because the fissure is not a flat surface and the effect of laser varies as the focal working distance varies. In this study, we used the standardized working focal distance of 12 mm by using a custom-made apparatus so that we could fix the laser handpiece and the samples had the free access to be moved on a stable platform. However, the distance between the handpiece and entrance, middle part, and bottom of fissure could not be standardized, so uniform ablation of the entire fissure surface was not possible and this could have been a great disadvantage of laser for the surface treatment of fissures. Since etching with laser system used a motion controlled by the hand, it resulted into uneven etching patterns. The SEM findings of Olivi *et al*.[16] were also confirmed by this study; wherein it showed cratering effect with uneven margins, melting with lava-like concrescences on irregular but homogeneous base [Figure 4b]. Areas of vetrification divided by grooves and cracks with disappearance of normal prismatic pattern probably due to thermal effect were seen, which might also have been the reason for poor adaptation in laser group.

Conclusion

Based on the results of the study, it may be concluded that:

1. The unfilled and filled sealants showed no significant differences in terms of penetration and adaptation. Hence, both seemed to behave in the same manner
2. The surfaces prepared by Er: YAG laser showed poor adaptation compared to those prepared conventionally and by fissurotomy with acid etching hence either of these two methods of enamel conditioning could be preferred.

Hence, from the above findings, we would like to suggest that the material properties *per* Se may not influence the sealant success. The type of the technique used to prepare the enamel surface appears to be one of the important factors to enhance a good seal at the sealant tooth interface. The conventional acid etching after the routine pumice prophylaxis seems to be a better treatment option before the application of sealants.

Clinical significance

The study would help the clinicians in choosing a right sealant material and the technique of enamel preparation.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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