RESEARCH ARTICLE

An *In Vitro* Microleakage Study for Comparative Analysis of Two Types of Resin-based Sealants Placed by Using Three Different Types of Techniques of Enamel Preparation

Poonam Shingare[1], Vishwas Chaugule[2]

Abstract

Background: Clinicians always experience dilemmas while choosing the type of pit and fissure sealant and the method of enamel preparation before the application of sealant. This study was accomplished using the unfilled and filled types of resin sealant deploying three different techniques of enamel preparation.

Aim and objective: To do a comparative analysis of unfilled and filled sealants by deploying three techniques of enamel preparation.

Materials and methods: The total number of 60 extracted teeth were divided into 3 groups, each containing 20 samples. Conventional acid etching of enamel was labeled as (group I), laser application as (group II), and fissurotomy bur (group III). The samples of group I were prepared by conventional acid etching, the group II was subjected to Er:YAG lasing, while in group III fissurotomy followed by acid etching was done. The sealant placement was done using split tooth design in all the samples. Dye penetration using 5% methylene blue was used for microleakage assessment.

Results: The highest microleakage was found with Gr. II whereas Gr. I exhibited the least microleakage. No statistical difference was observed between the unfilled and filled sealant ($p = 0.652$).

Conclusion: Conventional acid etching alone or with fissurotomy weighed up appropriate option regardless of the type of sealant material used.

Clinical significance: In regard to the selection of material and proper technique of enamel preparation, this study will be useful to clinicians.

Keywords: Acid etching, Enamel preparation, Fissure sealants, Lasing, Microleakage, Pit.

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Introduction

"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".[1] The representative properties of an ideal sealant material comprise biologically compatibility, sufficient bond strength, acceptable marginal integrity, abrasion and wear resistance, anticarcinogenicity, and economical.[2]

Bowen (1962) introduced Bis-GMA resin which leads to the development of resin sealant material which is the forerunner of currently available sealants.[3] For the advancement of the properties of the sealants filler particles, colors and fluorides have been added to them.

Retention of the sealant is very important for their clinical efficacy.[4] Factors that are responsible for retention are pit fissure morphology, proper isolation, enamel conditioning, application techniques, and material properties like surface tension and adhesion.[5] The penetrability of sealant is determined by the geometric configuration of fissures as well as by physiochemical characteristics and polymerization shrinkage of sealant. Sealant retention can be improved by using various methods like cleaning the fissure surface with hydrogen peroxide, pumice prophylaxis, air polishing, air abrasion, and fissure preparation with a bur. Deeper areas of fissure can be accessed by mechanically preparing the fissure with bur which permits evacuation of debris and allows penetration to deeper areas.[6]

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One more crucial factor deciding sealant success is microleakage. "Microleakage or marginal leakage may be defined as the ingress of oral fluids into the space between the tooth and restorative material".[7] Prevention of microleakage by tightly sealing the pit or fissure is important as it may favor caries underneath the sealant. Microleakage and marginal sealing ability of the material can be evaluated by *in vitro* studies.

Resin sealants are technique sensitive require proper isolation and avoidance of moisture contamination, failure to which cause microleakage. Use of new technique, i.e., LASER can overcome these detrimental steps. Laser with distinct properties are accessible nowadays and being utilized in various fields of dentistry. One among them is Erbium:Yttrium Aluminium Garnet laser (Er:YAG, wavelength = 2.94 μm). The use of Er:YAG laser is secure and efficacious for hard tissue treatments like cavity preparation, caries removal, and acid etching and has been approved by FDA.

There are conflicting results in the dental literature regarding the success of filled and unfilled resin-based sealants which make clinicians more confused in the selection of the most appropriate and suitable material. The ideal method of enamel conditioning before placing dental sealants or the use of no additional method before placing a dental sealant is quite a perplexing question. This study is an attempt to resolve such confusions in the minds of clinicians.

Aim and objective

Aim

To perform an appraisal of unfilled and filled resin sealants and analyze the use of different enamel conditioning techniques.

Objectives

The objectives of the study were:

- To do a comparative analysis of two pit and fissure sealants, i.e., filled and unfilled by studying—The microleakage at the tooth sealant interface.
- To evaluate and compare conventional acid etching, lasing, and bur technique of preparation of fissures by studying—The microleakage at the tooth sealant interface.

Materials and Methods

Methodology

The study was carried out using 60 teeth that were collected for the study purpose from the patients in whom extractions were indicated for an orthodontic purpose[8] or requiring removal of third molars.[9,10] Teeth that were free of caries, restorations in any form, fluorosis, developmental defects, hypoplasia, fractures, and cracks[11,12] were selected in the study, and teeth with abnormal morphology were excluded from the study.[10]

Soon after the extraction cleaning of teeth initially with water and then followed by hydrogen peroxide was carried out which was again followed by hand scaling.[12] Pumice prophylaxis with a rubber cup and bristle brush was done followed by rinsing with water[13–15] after which ultrasonic cleaning was done. Samples were then examined for caries if any, using a sharp explorer, and were stored in chloramine T solution until further use at room temperature.[14,16]

The samples were further divided into 3 groups, each containing 20 samples for conventional acid etching of enamel (group I), for treatment of enamel with laser (group II), and for treatment of enamel with fissurotomy bur (group III).

The occlusal surface of all the samples of group I was cleaned with water and dried.[17] Etching of teeth with 37% phosphoric acid gel was carried out according to the manufacturer's instruction[15] which were again washed[18,19] and dried.[12,17] Split tooth design was used for sealant application in all the samples. The design utilizes both the sealant materials in the same tooth, placed either on the mesial or distal half of the fissure.[15] The sealant application was completed according to the manufacturer's instructions. Sealrite (Pulpdent Corporation, Watertown, MA 02471-0780 U.S.A. 4.4% filled)/Clinpro (3M ESPE, Dental Products, St. Paul, MN 55144-1000, unfilled) was applied to either mesial/distal half of the fissure and cured using dental light cure unit (Hilus). After curing the first sealant second sealant was applied to the remaining mesial/distal half in the same manner and cured. Er:YAG laser in non-contact and scanning mode was used to perform lasing of the fissure surfaces of the samples in group II using 350 mJ energy at 6 Hz frequency and power of 2.1 Watt by keeping the standardized focal distance between the sample and the handpiece (R02) of 12 mm. The laser beam was directed at right angles to the occlusal surface. The middle portion of 1 mm width was kept unprepared.[20] Then sealant application was done by similar method which was used for group I (no acid etching done following laser treatment). Carbide fissurotomy bur (Fissurotomy Bur 18,010 for molars and 18,013 for premolars. SS White, Ivoclar North America, Inc.) was used to enlarge the fissure before doing acid etching in Gr. III using airotor hand piece in a light sweeping motion[16,21] middle portion of 1 mm width was kept intact.[15] After which acid etching, cleaning, and sealant application were done in similar way that of Gr I. Sealed containers were used to store all the samples from group I, II, and III for 1 week at 37°C.[8] After 1 week, the teeth were thermocycled at 5, 37, and 55°C using dwell time of 30 seconds for 500 cycles.[12,16,22] The samples were again stored for 1 week in saline at 37°C.[8]

Microleakage Examination

Sealing of the apices of samples was done with sticky wax,[16] followed by coating the surfaces with nail varnish using two different colors for mesial and distal half for identification of material leaving about 1 mm margin around the sealant. The samples were then immersed in 5% methylene blue solution for 4 hours.[8] The coatings were removed and the samples were washed and then embedded in acrylic resin blocks made with the help of L molds.[14] The samples were then sliced longitudinally in a mesiodistal direction[19,22] using double-sided diamond disc underwater coolant in such a way that each section contained both the materials in the mesial and distal fissure.[23] The sections thus obtained were polished with silicon carbide paper and diamond paste used for finishing.[24] 3% sodium hypochlorite was used for rinsing followed by washing with tap water and then dried. The examination was done under a stereomicroscope (Leica-MZ6 Trinocular stereozoom microscope) for microleakage assessment at 4× magnification. Each section was scored for microleakage according to the scoring system used by Overbo and Raadal.[7–9,25]

Microleakage score:
Score 0—no dye penetration (Fig. 1).
Score 1—dye penetration in the outer half of the fissure (Fig. 2).
Score 2—dye penetration in the inner half of the fissure (Fig. 3).
Score 3—dye penetration to the base of the fissure (Fig. 4).

Independent examination and evaluation were done by two different examiners.

Statistical Analysis

All statistical analyzes were done using SPSS. The arithmetic means of scores for microleakages were calculated in all the groups. Kappa statistics were used to determine the interexaminer variability. Statistical analysis was done by Kruskal–Wallis test, Mann–Whitney test, and Wilcoxon signed-ranks test at 95% confidence level.

Results

Table 1 shows the mean scores and standard deviations obtained for microleakage for Clinpro (unfilled sealant) in the three techniques

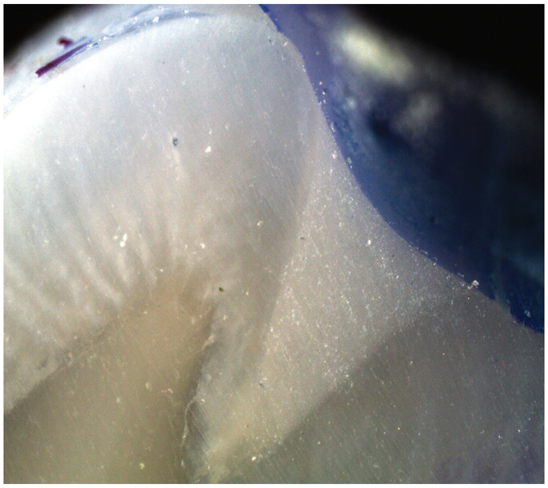

Fig. 1: Microleakage score 0—no dye penetration

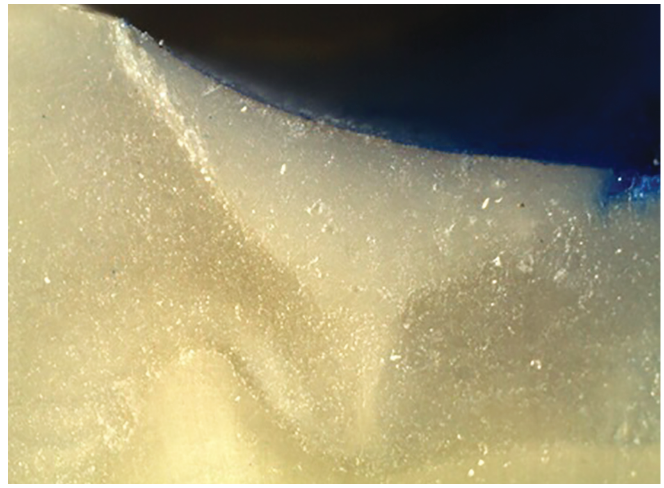

Fig. 2: Microleakage score 1—dye penetration in the outer half of the fissure

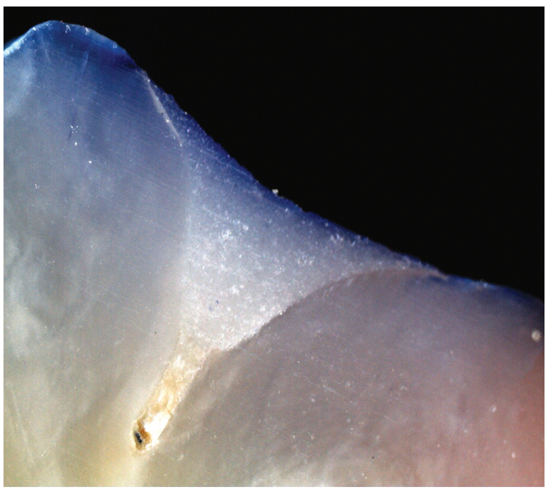

Fig. 3: Microleakage score 2—dye penetration in the inner half of the fissure

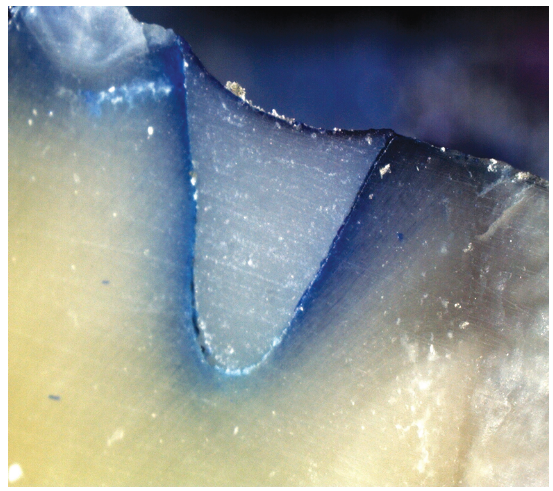

Fig. 4: Microleakage score 3—dye penetration to the base of the fissure

Table 1: Comparison of microleakage of clinpro (unfilled) sealant among three techniques

Group	*N*	*Mean*	*Std.[†] deviation*	*Kruskal–Wallis test χ^2 (df = 2)*	*p*
Acid etching (I)	40	0.57	0.747	16.07	0.001 HS***
Laser (II)	40	1.48	1.132		
Fissurotomy (III)	40	0.75	0.742		
				Mann–Whitney test *Z*	*p*
I vs II	40			3.7	0.001 HS***
I vs III	40			1.23	0.22 NS*
II vs III	40			2.90	0.004 Sig**

[†]Standard
*Statistically non-significant
**Statistically significant
***Statistically highly significant

of enamel treatment. The mean values for acid etching, laser, and fissurotomy were found to be 0.57, 1.48, and 0.75, respectively. Microleakage in acid etching technique was found to be the least when compared with laser and fissurotomy. Highest microleakage score was seen in laser group, followed by fissurotomy and acid etching (Fig. 5). Highly significant differences were reported with the Kruskal–Wallis test among the three groups with *p* value 0.001 ($p < 0.05$). Comparison of Gr. I and Gr. II showed highly significant difference ($Z = 3.7$ with *p* value of 0.001). Comparison of Gr. I and Gr. III showed statistically no significant differences ($Z = 1.23$ and the *p* value 0.22) ($p > 0.05$). Comparison of Gr. II and Gr. III showed significant differences ($Z = 2.90$, *p* value 0.04) ($p < 0.05$).

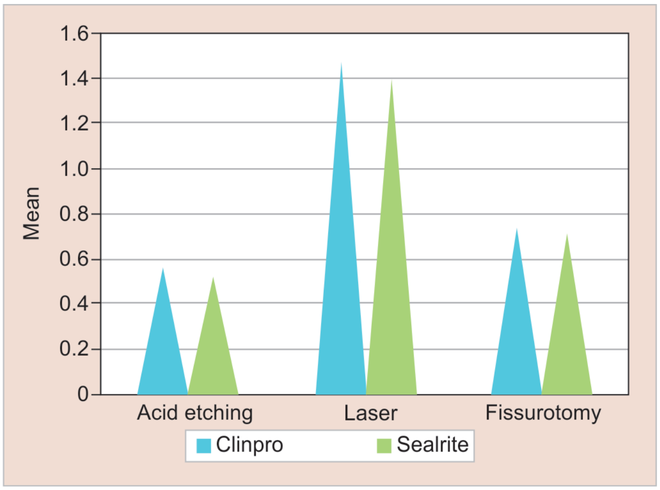

Fig. 5: Microleakage comparison of unfilled (Clinpro) and filled (Sealrite) sealant in three techniques of enamel preparation

Table 2 displays the values obtained for microleakage when Sealrite (filled sealant) was applied after treating the enamel by the three techniques. The observed mean values for acid etching, laser, and fissurotomy are 0.53, 1.43, and 0.70, respectively. Microleakage in acid etching is observed to be least when compared with laser and fissurotomy. Highest microleakage score is seen in laser group, followed by fissurotomy and acid etching (Fig. 5). The Kruskal–Wallis test is showing highly significant differences in microleakage among the three groups with p value of 0.001 ($p < 0.05$). The Mann–Whitney test shows highly significant differences for Gr. I *vs* Gr. II and for Gr. II *vs* Gr. III with p value of 0.001 ($p < 0.05$). For comparison of Gr. I and Gr. III, the Mann–Whitney test shows nonsignificant differences with p value of 0.24 ($p > 0.05$).

Table 3 and Figure 5 show the comparison of microleakage between Clinpro (unfilled) and Sealrite (Filled) sealant for acid etching group, laser group, and fissurotomy group, respectively. The difference between two materials was found to be statistically nonsignificant by the Wilcoxon signed-ranks test with p value being 0.707 ($p > 0.05$) in acid etching, 0.827 ($p > 0.05$) in laser

Table 2: Comparison of microleakage of sealrite (filled) sealant among three techniques

Group	*N*	*Mean*	*Std*[†]*. deviation*	*Kruskal–Wallis test* χ^2 *(df = 2)*	*p*
Acid etching (I)	40	0.53	0.554	19.02	0.001 HS***
Laser (II)	40	1.43	1.059		
Fissurotomy (III)	40	0.70	0.648		
				Mann–Whitney test *Z*	*p*
I vs II	40			4.0	0.001 HS***
I vs III	40			1.18	0.24 NS*
II vs III	40			3.21	0.001 Sig**

[†]Standard
*Statistically non-significant
**Statistically significant
***Statistically highly significant

Table 3: Microleakage comparison of clinpro and sealrite sealant in acid etching, laser, and fissurotomy group

Acid etching	*N*	*Mean*	*Std*[†]*. deviation*	*Wilcoxon signed-ranks test Z*	*p*
Clinpro	40	0.57	0.747	0.376	0.707 NS*
Sealrite	40	0.53	0.554		
Laser	*N*	*Mean*	*Std*[†]*. deviation*	*Wilcoxon signed-ranks test Z*	*p*
Clinpro	40	1.48	1.132	0.218	0.827 NS*
Sealrite	40	1.43	1.059		
Fissurotomy	*N*	*Mean*	*Std*[†]*. deviation*	*Wilcoxon signed-ranks test Z*	*p*
Clinpro	40	0.75	0.742	0.464	0.643 NS*
Sealrite	40	0.70	0.648		

[†]Standard
*Statistically non-significant

Table 4: Comparison of microleakage of clinpro and Sealrite irrespective of the technique of preparation

Microleakage	*n*	*Mean*	*Std*[†]*. deviation*	*Wilcoxon signed-ranks test Z*	*p*
Clinpro	120	0.93	0.968	0.45	0.652 NS*
Sealrite	120	0.88	0.871		

[†]Standard
*Statistically non-significant

group, and 0.643 ($p > 0.05$) in fissurotomy group. Table 4 shows microleakage comparison of clinpro (unfilled) and sealrite (filled) sealant irrespective of technique of preparation.

Discussion

Prevention of microleakage at the margins is of prime importance otherwise caries may continue beneath the sealant[26] especially in case of questionably carious fissures.[27] The clinician frequently encounters difficulty while choosing invasive or noninvasive techniques while placing a sealant. The study was designed to explore and correlate the microleakage appearing after the placement of filled and unfilled sealant by using three techniques of enamel surface preparation.

The surface attributes of enamel can be predicted to alter with change in enamel conditioning techniques so the material applied may perform differently with each technique. So we hypothesized that the filled sealant may behave differently with acid etching, lasing, and enameloplasty of the enamel fissure. Similarly, the unfilled sealant may behave differently. As the composition and material characteristics change the material behavior is expected to change with different enamel surface treatments. Therefore, it was decided to check the two materials (i.e., filled and unfilled sealants) and to provide the best combination of material and technique.

Despite various methods used to assess microleakage like zero resistance current method,[28] or dye recovery method. The use of dye penetration is an easy, simple, and extensively used procedure. Varied dyes and solutions are in use at the varying concentration for investigating microleakage such as methylene blue,[7] radioactive isotopes,[29] basic fuchsin,[30] erythrosine, silver nitrate,[31] alcohol gentian violet,[32] and rhodamine.[33]

Various techniques have been used to evaluate dye penetration through tooth sealant interface[34] but the use of a ranking scale to score dye penetration was common in most studies.[35]

The significant finding put forth from this study was that the conventional acid etching and fissurotomy with acid etching exhibited almost equivalent microleakage. This finding was in accordance with the finding by Boj et al.[36] who showed no statistical difference in sealant microleakage after bur or conventional preparation. Similar results were observed by Hatibovic-Kofman et al.[37] showing no significant difference in the two techniques. Mentes and Gencoglu[9] also found similar results with the bur and acid etching group displaying similar microleakage. Xalabarde et al.[38] too revealed a non-significant difference in microleakage for comparisons of enameloplasty and etching. Salama and Al-Hammad also reported congruous results.[18]

The outcomes of this study were contrary to those of Hatibovic-Kofman et al.[7] who reported less microleakage with fissurotomy plus acid etching than conventional acid etching and air abrasion tooth preparation. Similarly, Subramanian et al.[10] also found less microleakage in sealants placed using tooth preparation than conventional acid etching. Our results showed less microleakage in the acid etching group which was in disagreement with that of Algarni and Elkwatehy who showed there was more microleakage in the acid etching group than that without acid etching.[39]

The disparities in the finding may be due to different enamel preparations. Hatibovic-Kofman et al. opened the fissures to nearly the diameter of ¼ round bur in a low-speed handpiece. Halterman et al.[40] found that variations in the dimensions and the types of the material of instrumentation had a greater influence on the topography of enamel surface both at the micro and macroscopic levels. Various types of burs such as diamond tapered fissure Komet #8392, ¼ round bur, pear-shaped bur, Sorenson bur #2137, number 1 point, number 2 point, # 1 round steel bur, carbide #330, Sorenson Ponta K.G. 2137F, Komet #8833, ¼ stainless steel round bur, #582 S diamond bur, Fissurotomy bur 18010, and Diamond tapering fissure bur have been used by various researchers for mechanical preparation. The tapered fissure diamond point opens the fissures along the entire length of a fissure. The use of the Komet # 8392 diamond bur suites to the anatomic form of the fissures. In this study, we used light sweeping strokes for fissurotomy purposes to keep the unnecessary removal of the healthy tooth structure at a minimum. There is a need to explore the effect of various preparation techniques.

One additional thing that could have differed the results is that in our study after pumice prophylaxis we cleaned the fissures with an ultrasonic tip which removed all the debris and any remaining pumice which could have given better and comparable results with the fissurotomy preparation. Variations in selecting a technique may be dependent on the individual's choice.

Sealants placed in the laser prepared surfaces showed maximum microleakage than in the fissurotomy and acid-etched surfaces. These findings were similar to the findings reported in previous studies by Borsatto et al.,[41] do Rego and de Araujo,[42] Francescut and Lussi,[23] Youssef et al.,[31] Lupi-Pégurier et al.,[43] and Corona et al.[44] The results of Topaloglu-Ak et al. also reported that there was highest microleakage in laser etching group and least microleakage in laser combined with acid etching group.[45]

However, our results were in contrast with those of Moshonov et al.[46] and Jana et al.[47] who reported a similar degree of microleakage while comparing lasing and acid etching. This difference in findings might have been due to differences in the use of laser parameters or the use of different laser machines. The study utilized 350 mJ energy at 6 Hz frequency while a study by Moshonov et al. used 800 mJ energy at 12 Hz frequency while Jana et al. used 105 mJ energy. Other reasons that could be sighted were the use of different dyes for microleakage study and the use of different scoring systems to measure the microleakage. Our study deployed 5% methylene blue us while Jana et al. used 0.5% basic fuchsin. For measuring microleakage, we used an ordinal ranking score system by Overbo and Raadal.[25] Use of different sealants by different researchers could also be a factor contributing toward the variation in the final outcome. The scanning electron microscopic examination studies of the laser-treated fissured enamel surface reported "crater" shaped depressions which caused the uneven cracking appearance of the surface. Non-contact and discontinuous emission mode of laser results in diverse distribution of pits on the surface. The deeper fissures are expected to be stroked at most at their openings and the laser beam could not reach the bottom of the fissure.[23] This depends on working focal distance. A standardized working focal distance of 12 mm was utilized in this study with the use of custom-made apparatus made of acrylic resin. But uniform ablation of the entire fissure surface was not possible because fissure is not a flat surface. Laser etching uses hand-controlled motion causing a non-uniform etching pattern.

The SEM findings of Giovanni et al.[20] showed a "cratering effect with uneven margins, melting with lava-like concrescences on irregular but homogeneous base. Areas of vitrification divided by grooves and cracks with the disappearance of normal prismatic pattern probably due to thermal effect were seen, the reason for more microleakage in the laser group."

Literature shows contrasting results from various microleakage studies comparing unfilled and filled sealants. Our study showed that both filled and unfilled resin sealants had similar microleakage. The results of this study were consistent with previous findings that showed similar results for both the sealants by Park et al.[48] and Xalabarde et al.[49] Contrary to the reports of Hatibovic-Kofman et al.[37] which showed unfilled sealant having less microleakage than filled sealant this study exhibited the otherwise results.

Inconsistent reports of various microleakage studies could be attributed to the use of a variety of methods deployed to study microleakage like the use of different dyes in different concentrations, use of various scoring systems. Varying use of thermocycling, different regimes of thermocycling could be cited for diverse results. This could have been the reason for different results.

Conclusion

- The unfilled and filled sealants showed similar behavior when investigated for microleakage.
- Er:YAG laser etching for enamel surface preparation exhibited greater microleakage than conventional acid etching and fissurotomy with acid etching.
- Conventional acid etching and fissurotomy with acid etching techniques were performed similarly in the prevention of microleakage. Any one of the methods of enamel conditioning can be preferred.

So from the above finding, we would like to suggest that the material properties do not influence the sealant success, and also the penetration of the sealant is not much important than its adaptation giving a sealed interface to prevent microleakage. The tight seal between the sealant tooth interfaces depends on the type of technique used to prepare the surface. The conventional acid etching after the routine pumice prophylaxis seems to be a better treatment option to apply sealants.

The laser can become a good alternative for enamel treatment before sealant application but it has a long way to go through more research so that definite parameters can be set for the laser etching in the future but for today we suggest that "OLD IS GOLD" that is the conventional acid etching could be the preferred option.

Clinical Significance

The results of the study will help clinicians to select the best material from a large number of the commercially available pit and fissure sealants. Also, it will clear the doubts regarding the selection of the method of enamel preparation before sealant application. Conclusions of this study show that there is a great amount of scope for future studies on lasers so that suitable criteria can be set for laser etching.

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