**Archives of Orofacial Sciences**
**The Journal of the School of Dental Sciences, USM**

*Arch Orofac Sci* (2017), **12**(1): 12-22.

**Original Article**

# *In-vitro* comparative study of marginal leakage and penetration ability of moisture-tolerant and conventional resin-based pit and fissure sealants with different surface preparations

Thavamalar Marimuthoo[a], Nor Atika Md Asfar[b], Mariati Abd. Rahman[b], S. Nagarajan M.P. Sockalingam [a]*

[a] Department of Operative Dentistry, [b] Department of Clinical Oral Biology, Faculty of Dentistry, Universiti Kebangsaan Malaysia (UKM), Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia.

* Corresponding author: drnaga67@gmail.com

Submitted: 21/10/2016. Accepted: 15/02/2017. Published online: 15/02/2017.

**Abstract** This *in-vitro* study aimed to evaluate and compare the marginal leakage and penetration ability of a moisture-tolerant (Embrace WetBond™) and a conventional (Clinpro™) resin-based sealants under three different enamel surface preparations (acid etched, acid etched and saliva contaminated and bur preparation and acid etched). One hundred and twenty extracted caries free human premolars teeth were cleaned and randomly divided into six groups of equal numbers, according to the type of sealants used and surface preparations. All the sealed teeth were subjected to thermocycling and immersed in a methylene blue dye. Each tooth was then embedded into acrylic resin before it was sectioned into four sections per tooth. Marginal leakage and unfilled surface area (indicating penetration depth of resin) were then measured using an optical 3D measurement device (Alicona Infinite Focus®). Both sealants exhibited comparable proportion of marginal leakage on acid etched only surfaces. Moisture-tolerant sealant showed the least proportion of marginal leakage on bur prepared and etched surfaces. Presence of saliva has detrimental effect on adhesion of both sealants. Nevertheless, depth of penetration of sealant into the fissures is comparable with both sealant types irrespective of the surface preparations.

**Keywords:** Hydrophilic sealant, marginal leakage, penetration ability, pit and fissure sealant.

## Introduction

Global trend shows dental caries has reduced remarkably over time because of caries preventive effects of various fluoride agents and increased awareness of oral health knowledge. Despite this, caries reduction was only noted significantly at the least susceptible sites (smooth and interproximal surfaces), while the most susceptible site (occlusal) recorded the smallest reduction (McDonald and Sheiham, 1992; Mejàre *et al.*, 1998). This necessitates the use of pit and fissure sealant to avert the onset of early caries lesion on occlusal surfaces as first suggested by Cueto and Buonocore (1967) who despite being restricted by materials science, had demonstrated that after 1 year, the use of an enamel adhesive to seal the pits and fissures of 601 caries-free molars and premolars without cavity preparation resulted in an 86.3% caries reduction compared with similar number in the controlled teeth. Since then various studies have been conducted to further improvise this technique in terms of the types of material used and its composition, enamel surface preparations as well as placement methods (Hatibovic-Kofman *et al.*, 2001; Burrow *et al.*, 2003; Khogli *et al.*, 2013; Godhane *et al.*, 2015).

There are two main types of sealant materials available commercially, namely the resin-based and glass-ionomer cement sealants. However, resin-based sealant is often the preferred choice (Ahovuo-Saloranta *et al.*, 2013). Sealants can be polymerized either by auto-polymerization or light-cured and some have incorporated fluoride in them to further enhance their property in caries prevention. In spite resin sealants been widely used, one of their main drawbacks is moisture sensitivity and their success very much dependent on obtaining an optimum moisture control. This can be very challenging to achieve especially in young patients or if the tooth is partially erupted.

In recent years, there has been a significant advancement in resin-based sealants especially with regards to the development of moisture-tolerant chemistry. This move is to overcome the shortcomings of traditional sealants, which were hydrophobic. One of such products is the Embrace WetBond™ (Pulpdent®, Watertown, MA) which was claimed to be a hydrophilic, moisture-tolerant resin-based sealant that does not require an additional bonding agent. Upon light curing, the polymerized sealant of the Embrace WetBond™ has been claimed to have the physical properties similar to other commercially available sealants (Murnseer *et al.*, 2007). Since much of the dental caries prevention is focused during early childhood period where moisture control can be a significant problem, the invention of moisture-tolerant fissure sealant is believed to give a better and alternate option to the glass ionomer cement in sealing pits and fissures under non-optimal conditions. It also may provide the dental practitioners an alternative approach to the wait and watch approach on partially erupted teeth. Although a 2-year clinical study showed 95% clinical success with Embrace WetBond™ (Strassler and O'Donnell, 2008), nevertheless, to date there is still a gap in the information as to whether moisture-tolerant material can provide adequate marginal sealing and penetration as compared to conventional resin-based sealant, which is commonly used under normal circumstances and proven to be effective.

The objective of the present study was to evaluate and compare the marginal leakage and penetration ability between a moisture-tolerant (Embrace WetBond™, Pulpdent®, Watertown, MA) and a conventional (Clinpro™ Sealant, 3M ESPE®, St.Paul, USA) resin-based sealants under three enamel surface preparations, as well as to compare within same sealant groups under different enamel surface preparations. It is believed that the outcome of this may shed some important insight to dental practitioners on selection of sealant to be use in their clinics.

## Materials and methods

The study was conducted at the Faculty of Dentistry, Universiti Kebangsaan Malaysia (UKM). Prior to the commencement of the present study, an ethical approval was obtained from the UKM ethics committee [1.5.3.5/244/DD/2011/044(1)]. One hundred and twenty caries free human maxillary premolar teeth, extracted for orthodontic purposes were utilised in the present study. The maxillary premolars were chosen to standardise the fissure pattern variations that might occur if the lower premolars were to be included. Based on Khogli *et al.* (2013) study, the number of teeth needed for the current study was calculated using a sample size formula (Fig. 1). The extracted teeth were cleaned and stored in a normal saline filled plastic container to prevent dehydration until they are ready to be used. Teeth with previous sealants or restorations and structural defects were excluded from the present study.

### *Preparation and sealant application of the teeth*

At the time of research, crowns of the selected teeth were cleaned using prophylaxis brush on a slow-speed handpiece and the fissures were removed of debris using a Probe 9. Then, the teeth were rinsed with distilled water using air-water spray and dried with oil-free compressed air. Thereafter, the teeth were randomly assigned into six groups (Group 1-6) in equal numbers with 20 teeth in each group (Table 1).

The occlusal fissures of teeth in Group 1 and 2 were sealed based on the respective manufacturers' instructions and used as control groups. The occlusal fissures were etched with etchants of 35% and 38% phosphoric acid respectively for 15 seconds. The fissures were then thoroughly rinsed with distilled water and dried with a gentle stream of oil-free air until the etched surface appeared as matte frosty white for teeth in Group 1. Thereafter, the Clinpro™ resin sealant was applied with a ball-ended burnisher into the etched pits and fissures and light-cured for 20 seconds with a halogen light curing unit at 500mW/cm$^2$ (Curing Light QHL75, Dentsply, USA). As for the teeth in Group 2, the occlusal fissures were thoroughly rinsed with distilled water and dried with cotton pellets to achieve a slightly moist status with no visible water. The Embrace Wetbond™ sealant was then placed over the fissures using a ball ended burnisher and light-cured for 20 seconds as per teeth in Group 1.

For Group 3 and 4, the occlusal fissures of the teeth were similarly etched, rinsed and dried as described for the Group 1 and 2 respectively. Subsequently, each tooth from the Group 3 was submerged into the artificial saliva prepared in a laboratory, with a composition similar to that described by Shannon *et al.* (1977) for 5 seconds and gently blow dried with a stream of oil-free air for 5 seconds before the Clinpro™ was applied and light-cured as per in Group 1. After submerging each tooth from Group 4 into the artificial saliva for 5 seconds, the occlusal fissures were lightly dried using a cotton pellet before the Embrace Wetbond™ sealant was placed over the fissures using a ball ended burnisher and light-cured for 20 seconds as per teeth in Group 2.

Finally, the occlusal fissures of the teeth in Group 5 and 6 were prepared with a tapered diamond bur, Komet 889M (Komet Dental, Gebr.Brasseler GmbH & Co, Lemgo, Germany) for micro-preparations without deepening them. Thereafter the fissures were etched and sealed with Clinpro™ and Embrace Wetbond™ sealants as per the manufactures' instructions similar to that of Group 1 and 2 respectively.

### *Processing of the samples*

The teeth from all of the groups were then stored in artificial saliva at 37°C for 1 week to simulate the oral condition. Artificial saliva was used instead of human saliva to overcome the issues related to infection control. After a week, the teeth were thermocycled at 5°C and 55°C in thermocycling waterbath (AMMP Centre, Kuala Lumpur, Malaysia) for 500 cycles, with a dwell time of 20 seconds and transfer time of 2 seconds between baths, after which all teeth were rinsed with an air-water spray and dried with compressed air.

All teeth were then coated with two layers of nail varnish approximately 1 mm from the fissure sealant area and apices were sealed with sticky wax. All teeth were immersed in 5% methylene blue for four hours, after which they were rinsed thoroughly under tap water. Each tooth was then embedded into self-cured clear acrylic and sectioned longitudinally in a buccolingual direction through the line connecting the buccal and palatal/lingual cusp tip using a water-cooled diamond impregnated linear precision saw, Isomet 4000 (Buehler, Illinois, USA). Four sections (two lateral sections and two central sections) from each tooth were obtained for the evaluation of marginal leakage and depth of sealant penetration and this will provide 80 tooth sections in each group for analysis. However, it was expected that some of these sections may not be suitable for analysis due to technical and processing errors. In line with the sample size calculation, a minimum number of 64 analysable tooth sections in each group would be adequate to guarantee a power of 80% for the reliability of the study.

### *Assessment of marginal leakage and penetration ability*

Image of each tooth was captured (Fig. 2) and then assessed for the marginal leakage and penetration ability, using an optical 3D measurement device (Alicona® Infinite Focus, Alicona Imaging GmbH, Austria). The evaluation of the marginal leakage and penetration ability were based on the method described by Khogli *et al.* (2013). The marginal leakage was evaluated quantitatively using the formula [(a+b) / (c+d)] (Fig. 3). Meanwhile, the penetration ability was assessed as the proportion of area of the fissure which was unfilled by the sealant relative to the whole fissure area using the formula [f / (e+f)] (Fig. 4). Three readings of marginal leakage and penetration ability were taken for each sample and the mean value were calculated and compared to assess for the measurement error.

### *Intra-examiner reliability*

The measurements for the marginal leakage proportion and penetration ability of the sealants were reassessed after 2 weeks by the same examiner in order to determine the intra examiner reliability. One-tenth (10%) of the sample sizes were randomly selected for this purpose. Paired t-test was used to evaluate the differences between the actual measurement and the repeated measurement. There were no significant differences in both the marginal leakage and penetration ability scores when the measurements were repeated ($p<0.05$).

### *Data analysis*

The results were tabulated and analysed using the IBM SPSS Data Editor Version 20.0. Data were primarily computed to assess the distribution. Non-parametric tests, Mann-Whitney (comparison between two sealants on similar surface preparations) and Kruskal-Wallis (comparison between sealant groups with three different surface preparations) were used in the statistical analysis. The level of significance was set at $p<0.05$.

## Results

After tooth sectioning, 421 tooth sections were available for the marginal leakage and penetration ability analysis. Each group had more than minimum 64 analysable sections required for evaluation.

### *Comparison between sealants*

Table 2 revealed a lower proportion of marginal leakage for the conventional sealant (mean rank: 62.80) compared to the moisture-tolerant sealant (mean rank: 74.72) when the sealant application was done according to manufacturers' protocol. However, this was not statistically significant ($p>0.05$). Similarly, the difference in microleakage proportions between the conventional resin sealant (mean rank: 64.09) and the moisture-tolerant resin sealant (mean rank: 74.91) was not statistically significant when these sealants were placed under saliva contamination ($p>0.05$). However, on the bur prepared fissures, the moisture-tolerant sealant (mean rank: 63.97) produced a statistically significant lesser marginal leakage ($p<0.05$) compared to the conventional resin sealant (mean rank: 81.66).

When compared between the sealant types and surface preparations in relation to penetration ability, both the moisture-tolerant and conventional resin-based sealants showed no statistical significant differences ($p>0.05$) (Table 3).

### *Comparison between the different surface preparations and each sealant type*

When the marginal leakage was compared between the different surface preparations and the conventional resin-based sealant (Clinpro$^{TM}$), there was a significant difference in at least one pair (Table 4). Pair-wise comparison showed that the control group (Group 1) presented with the lowest proportion of marginal leakage with statistically significant difference when compared with Group 3 ($p< 0.05$) as shown in Table 5. Similarly, for the moisture-tolerant sealant, comparison between the different surface preparations also showed a significant difference in at least one pair (Table 6). Pair-wise comparison revealed Group 6 had the lowest marginal leakage and Group 5 presented with the highest degree of marginal leakage (Table 7). Regarding the proportion of unfilled surface areas that reflected the penetration ability of the sealants, no statistically significant differences were noted with both sealants in all the surface preparation types (Table 8 and 9).

## Discussion

Sealant placement accounts for one of the many procedures undertaken for prevention of dental caries. Sealants are often placed in deep pits and fissures of teeth that accounts for 80% of caries seen clinically. Many sealant materials are marketed commercially and have said to be effective as caries preventive material (Godhane *et al.*, 2015). Sealants are expected to yield excellent result when they are placed in an ideal clinical setting. However, to obtain an ideal clinical setting is no easy means especially in children or partially erupted teeth.

In the present study, two types of pit and fissure sealants were tested for their surface integrity (marginal leakage measurement) and penetration ability when both were applied according to their respective manufacturers' instructions. When tested on acid etched and non-invasive enamel preparations, no statistical significant differences in the proportion of marginal leakage between the conventional and moisture-tolerant resin-based pit and fissure sealants. This suggests that both sealants are equally effective under ideal conditions on non-prepared fissures. Based on these findings, the moisture-tolerant resin based sealant appeared to be an appropriate sealant type to be used in situations where complete moisture control is impossible to achieve in patients. Few existing studies compared the conventional and moisture-tolerant sealants previously; however, they have mixed findings (Kane *et al*., 2009; Khogli *et al.*, 2013). These could be due to the diversity in the methodology used and/or use of different types of conventional sealants.

Both the scanning electron microscope (SEM) and optical stereomicroscope had been used widely in marginal leakage studies and shown to be effective in providing quantitative measurements that are necessary to evaluate the degree of marginal leakage (Soares *et al.*, 2005; Singla *et al.*, 2011; de Santi Alvarenga *et al.*, 2015). In the present study, quantitative measurements were obtained using an optical 3D measurement device (Alicona® Infinite Focus). This device is an optical stereomicroscope which provides better accuracy in term of detecting marginal leakage compared to other conventional qualitative scoring methods (Khogli *et al.*, 2013). The optical 3D measurement device allows visualization of the specimen in its actual visual appearance and allows measurement of specimen to be taken immediately at a high resolution. Thus, the true colour of dye penetration in the samples could be seen at its actual depth.

The moisture-tolerant resin-based pit and fissure sealant, Embrace WetBond™ was mentioned to have a unique chemistry that incorporates di, tri- and multi-functional acidic acrylate monomers in a formula with a hydrophilic-hydrophobic balance, thus it behaves favourably in moist oral environment. However, no statistical significant differences were noted in the present study concerning the proportion of marginal leakage and penetration ability on saliva-contaminated preparations when compared with the conventional resin-based hydrophobic sealant. This is because the excess saliva in the fissure was removed either by air-drying or with dry cotton pellet prior to the sealant placement. The results would have been different if the sealants were placed on saliva-filled fissures. However, this application would go against the manufacturers' protocol and may have yielded a negative outcome.

Invasive sealing technique has been introduced to improve the performance of pit and fissure sealants and it has been proven effective especially with the moisture-tolerant sealant. In the present study, when the fissures were widened using a tapered diamond bur, significantly better result was noted in terms of marginal leakage for the moisture-tolerant resin sealant compared to the conventional resin-based sealant. Khogli *et al*. (2013) also noted that superior results were only found in the groups sealed with moisture-tolerant resin sealant when fissure preparation with bur was done. This finding suggests that increase in the surface area may have allowed the moisture-tolerant resin-based sealant to adhere better to the prepared surfaces.

Concerning different surface preparation, it was noted in the current study that no statistical significant difference in the proportion of microleakage was noted between the acid etching preparation alone (control) and the bur prepared acid etching preparations for the conventional resin-based sealant. This agrees with the findings of Hatibovic-Kofman *et al.* (2001). Similarly, Khogli *et al.* (2013) also found both Er:YAG laser and bur preparation of enamel showed no statistical significant difference when compared to the conventional non-invasive sealing technique. Clinical studies on the advantages of bur preparations are also inconsistent. Shapira and Eidelman (1982) found in their clinical study that after one year there was no statistical difference in the retention of the sealants placed after conventional and bur preparations. Nevertheless, the same authors reported in their long-term three and six-year studies, bur preparations were found to be superior to the acid etching only (Shapira and Eidelman, 1986). On the other hand, the saliva-contaminated surface produced a statistically significant proportion of microleakage with conventional sealant compared to the control. One possible explanation to this is that the viscous saliva might have filled up the micro-tags created by etching and it is not effectively removed during drying process before the sealant placement. This in turn reduced the penetration of the resin into the micro-tags that permit mechanical retention, thus allowing space at the resin and fissure surface interface.

Interestingly, the proportion of marginal leakage was found to be the least in bur prepared fissure and acid etched preparation of the moisture-tolerant resin-based sealant when compared to the acid etching surface alone and the saliva-contaminated preparations. Khogli *et al*. (2013) also reported a similar finding. It is probably because of the property of moisture-tolerant resin filled sealant that enables the bulk to adapt and adhere strongly to the enamel surface and withstand the thermal stress of the oral environment. Clinically higher retention rates after mechanical pit and fissure preparation were reported to be attributed to superior penetration and increased bulk of the sealant (Shapira and Eidelman, 1986). This could be easily achieved by surface preparation of the fissure with a bur.

Burrow *et al.* (2001) had shown that the region just below the entrance of fissures were resistant to acid etching due to the presence of prismless enamel at the surface of the walls. They suggested that the removal of this enamel layer to allow formation of greater depth of micro-tags following acid etching, thus, increasing the area for adhesion. Since hydrophilic fissure sealant is a filled sealant with higher molecular weight and viscosity, an increase area of adhesion should provide a better retention. This probably explains the reduced proportion of microleakage found amongst the fissure-prepared samples sealed with hydrophilic sealants.

Penetration is an important factor in sealant application as it also determines the retention. To achieve good penetration, a sealant should be able to wet and flow even into tiny cresses. However, it is reported that the higher the viscosity, the lower the penetration of the material (Simonsen and Neal, 2011). In the present study, interestingly no statistical significant differences were noted in all circumstances. This could be probably due to the use of premolar teeth in the present study, as most premolars do not present with complicated fissures. Nevertheless, other studies also reported similar results even with the use of molar teeth (Xalabarde *et al.*, 1996; Khogli *et al.*, 2013).

## Conclusion

The current study showed that both the conventional resin based and moisture-tolerant resin based sealants are comparable in relation to the proportion of marginal leakage and depth of penetration if were to be used as per manufacturers' instructions on unprepared etched surfaces. However, presence of saliva may cause inadequate penetration of resin into micro-tags form for mechanical retention and can lead to failure. Since moisture-tolerant resin is a filled resin compared to the conventional resin that is unfilled, surface preparation of fissure with a bur can increase the total surface for penetration and adhesion, thus allowing better retention.

**Table 1** Description of the experimental Group 1-6 and composition of sealant materials

| Group | N (section) | Material | Manufacturer | Composition | Enamel pre-treatment | Surface condition |
|---|---|---|---|---|---|---|
| 1 | 71 | Clinpro™ | 3M ESPE | • Resin-based (Bis-GMA TEGDMA)<br>• Unfilled<br>• Fluoride release | Acid etching (phosphoric acid 35%) | Dry |
| 2 | 65 | Embrace WetBond™ | Pulpdent | • Resin-based (di-, tri-, and multifunctional acrylate) in an acid integrating network<br>• Filled sealant<br>• Fluoride release | Acid etching (phosphoric acid 38%) | slightly moist |
| 3 | 71 | Clinpro™ | 3M ESPE | Same as in Group 1 | Acid etching (phosphoric acid 35%) | Dry (saliva contaminated) |
| 4 | 69 | Embrace WetBond™ | Pulpdent | Same as in Group 2 | Acid etching (phosphoric acid 38%) | Slightly moist (saliva contaminated) |
| 5 | 74 | Clinpro™ | 3M ESPE | Same as in Group 1 | Fissure preparation with diamond bur and acid etching (acid phosphoric 35%) | Dry |
| 6 | 71 | Embrace WetBond™ | Pulpdent | Same as in Group 2 | Fissure preparation with diamond bur and acid etching (acid phosphoric 38%) | Slightly moist |

**Table 2** Comparison of marginal leakage between conventional resin-based sealant (Clinpro$^{TM}$) and moisture-tolerant resin sealant (Embrace WetBond$^{TM}$) on similar surface preparations

| Group | N | Mean Rank | *p*-value |
|---|---|---|---|
| 1 | 71 | 62.80 | 0.072 |
| 2 | 65 | 74.72 | |
| 3 | 71 | 64.09 | 0.111 |
| 4 | 69 | 74.91 | |
| 5 | 74 | 81.66 | 0.010* |
| 6 | 71 | 63.97 | |

* *p*-value < 0.05

**Table 3** Comparison of penetration ability between conventional resin-based sealant (Clinpro$^{TM}$) and moisture-tolerant resin sealant (Embrace WetBond$^{TM}$) on similar surface preparations

| Group | N | Mean Rank | *p*-value |
|---|---|---|---|
| 1 | 71 | 67.18 | 0.640 |
| 2 | 65 | 69.95 | |
| 3 | 71 | 71.06 | 0.846 |
| 4 | 69 | 69.92 | |
| 5 | 74 | 74.92 | 0.323 |
| 6 | 71 | 68.96 | |

* *p*-value < 0.05

**Table 4** Comparison of marginal leakage of the conventional resin-based sealant (Clinpro$^{TM}$) on different surface preparations

| Proportion | Mean Rank | | | *p*-value |
|---|---|---|---|---|
| | Group 1 | Group 3 | Group 5 | |
| Marginal leakage | 89.82 | 130.32 | 103.19 | 0.001* |

* *p*-value < 0.05

**Table 5** Pair-wise comparison of marginal leakage proportions of the conventional resin-based sealant (Clinpro$^{TM}$) between different surface preparations and their control

| Comparison groups | *p*-value |
|---|---|
| Control and saliva contaminated | 0.001* |
| Control and fissure prepared | 0.104 |

* *p*-value < 0.05

**Table 6** Comparison of marginal leakage of the moisture-tolerant resin-based sealant (Embrace WetBond™) on different surface preparations

| Proportion | Mean Rank | | | *p*-value |
|---|---|---|---|---|
| | **Group 2** | **Group 4** | **Group 6** | |
| Marginal leakage | 93.92 | 142.77 | 72.66 | 0.001* |

* *p*-value < 0.05

**Table 7** Pair-wise comparison of marginal leakage proportions of the moisture-tolerant resin-based sealant (Embrace WetBond™) on different surface preparations and their control

| Comparison groups | *p*-value |
|---|---|
| Control and saliva contaminated | 0.001* |
| Control and fissure prepared | 0.012* |

* *p*-value < 0.05

**Table 8** Unfilled area proportions of the conventional resin-based sealant (Clinpro™) on different surface preparations

| Proportion | Mean Rank | | | *p*-value |
|---|---|---|---|---|
| | **Group 1** | **Group 3** | **Group 5** | |
| Unfilled surface area | 108.30 | 105.15 | 110.48 | 0.842 |

* *p*-value < 0.05

**Table 9** Unfilled area proportions of the moisture-tolerate resin-based sealant (Embrace WetBond™) on different surface preparations

| Proportion | Mean Rank | | | *p*-value |
|---|---|---|---|---|
| | **Group 2** | **Group 4** | **Group 6** | |
| Unfilled surface area | 108.61 | 100.75 | 98.56 | 0.480 |

* *p*-value < 0.05

$$N = 2 \left[ \frac{(Z_{1-\alpha/2} + Z_{1-\beta})\, S_d}{\delta^2} \right]^2$$

N = Number of tooth sections
Z = The standard normal deviate for a one or two sided
$\alpha$ = Type I error
$\beta$ = Type II error
$S_d$ = Standard deviation
$\delta$ = A clinically acceptable margin

**Fig. 1** Formula used for calculation of sample size.

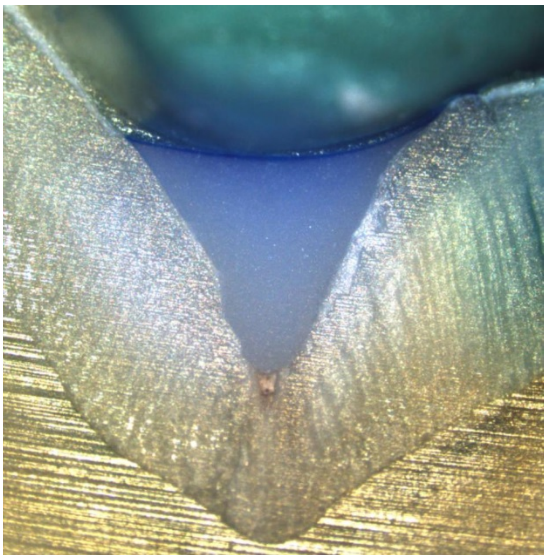

**Fig. 2** Captured image by the optical 3D measurement device showing penetration of fissure sealant into a fissure.

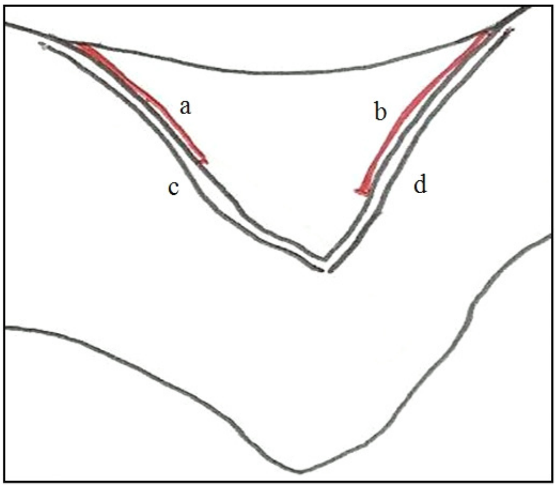


**Fig. 3** Diagram showing cross section of fissure with (a) and (b) representing the length of marginal leakage and (c) and (d) representing the length of sealant. Marginal leakage was assessed by dividing the total length of the leakage over the total length of tooth-sealant interface.

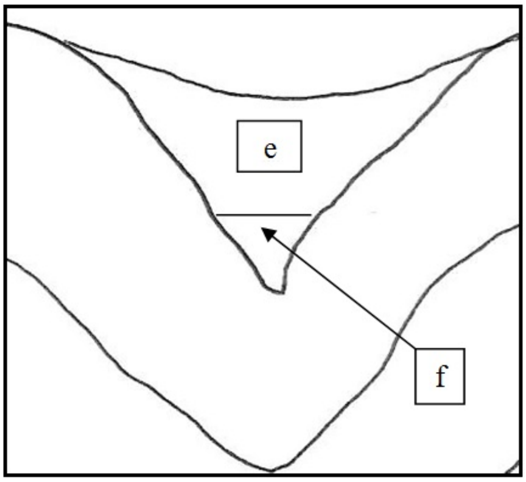


**Fig. 4** Diagram showing cross section of fissure with fissure sealant area as (e) and unfilled area as (f). Penetration ability was assessed as the proportion of area of the fissure which was unfilled by the sealant (f) relative to the whole fissure area (e+f)

## Acknowledgements

The authors like to wish to express the gratefulness to the all those involved in the successful completion of this research.

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