Comparisons of in vitro Penetration and Adaptation of Moisture Tolerant Resin Sealant and Conventional Resin Sealant in Different Fissure Types

Ramya Radhakrishnan IYER[1], Ajithkrishnan Champettil GOPALAKRISHNAPILLAI[1], Thanveer KALANTHARAKATH[1]

Objective: *To compare the penetration and adaptation of a moisture tolerant resin-based sealant with a conventional resin-based sealant in different occlusal fissure types in vitro by scanning electron microscopic (SEM) technique.*

Methods: *Fifty sound, intact extracted premolars and third molars were included. They were randomly and equally allocated to receive either Seal-Rite (conventional resin-based sealant) or Embrace WetBond (moisture-tolerant resin-based sealant). Etching and sealant applications were performed as per manufacturers' instructions. Specimens were sectioned at the deepest part of the fissure and viewed under SEM. Under SEM, fissure types were classified as U, V, I, IK or inverted Y. SEM images were analysed using Biowizard image analysis software to measure penetration and adaptation. The data were statistically tested.*

Results: *U fissures showed the highest mean percentage penetration. V forms exhibited the best adaptation. IK forms showed the poorest percentage penetration and adaptation. Fissure form significantly affected adaptation but not penetration. Embrace WetBond penetrated better than Seal-Rite into all the fissures but adapted excellently only in U fissures.*

Conclusion: *Fissure morphology significantly affected sealant adaptation. Moisture-tolerant Embrace WetBond was better than conventional Seal-Rite in penetration and adaptation into fissures.*

Key words: *occlusal fissure form, moisture-tolerant sealant, resin-based sealant*

With continuing caries reduction by various fluoride delivery systems and an increased focus on oral hygiene maintenance, the world has not seen much success in the prevention of pit and fissure caries. Today, caries is more and more a disease of the fissured surfaces. The morphological complexity of the pit and fissure system of posterior teeth accounts for their vulnerability to development of dental caries. Pits and fissures offer a much more favourable surface conditions for caries compared to knife-edged grooves and broad fossae, as these areas are sites of plaque and debris accumulation and least accessible to toothbrush bristles; bacteria are able to breed in the deep narrow defects where enamel did not form (called non-coalescence of enamel). Nagano observed that fissure morphology was highly varied and that there were fissure types that varied in forms, some of which were too narrow that would not permit even the entry of an explorer. He also elucidated that fissure morphology was a major consideration in the developmental pattern of pit and fissure caries[1]. The susceptibility of pits and fissure surfaces to caries challenged the prevailing techniques and technologies of prevention.

In the battle against decay in pits and fissures, there has not been a stronger warrior than the pit and fissure sealant. Sealant application is a conservative preventive measure that can be accomplished without anaesthesia

1 Department of Public Health Dentistry, KM Shah Dental College and Hospital, Sumandeep Vidyapeeth, Piparia, Vadodara, India.

Corresponding author: Dr Ramya Radhakrishnan Iyer. Department of Public Health Dentistry, KM Shah Dental College and Hospital, Sumandeep Vidyapeeth, Piparia, Vadodara. #102, Narayan Heaven; 37, Shukla Nagar, New Sama Road, Vadodara, Gujarat 390024, India. Tel: 91-9725036434; 91-0265-6539046; E-mail: ramya83r@rediffmail.com

or drilling of the tooth structure. Sealants are 100% effective against caries when they are fully retained. When used in a preventive dental programme in conjunction with water fluoridation, the effectiveness of water fluoridation increases by 20%[2]. The advantages of occlusal sealing include the decrease of caries risk up to nine times as compared to non-sealed teeth and the lower cost compared to the placement of restorations[3]. The preventive benefits of such treatments, however, rely on the sealant's ability to thoroughly fill pits and fissures and not to prematurely detach either partially or completely from occlusal surfaces. This implies that a sealant material must not only have good sealing ability but also have adequate longevity. The prime factors governing the life expectancy of a sealant are penetration into the fissures and adaptation to the walls of the fissures[4]. Apart from endowing retention to sealants, a well-penetrated fissure sealant is also desirable in order to reduce caries development at the deep crevice. Also, a deeply penetrated sealant is protected from shear forces occurring as a result of masticatory movements[5].

Penetration and adaptation rely on good clinical technique. Clinical evidence suggests that sealant loss (retention failure) within the first six months of placement is most likely due to inadequate moisture control[6]. Moisture contamination during sealant placement has hence been considered to be a hazard to sealant retention. Hence sealant materials with reasonable tolerance to moisture can be thought to contribute positively to long-term retention.

Choice of sealant materials becomes critical in a setting such as a community-based programme. Of all the currently available sealants, resin-based sealants are the most time-proven and have the highest retention rates that have been well tested and confirmed through longitudinal studies[7-10]. However, usually in public health programmes, lesser and more variable retention rates of sealants have been reported owing to unsatisfactory moisture control achieved in field conditions[6]. Failure of sealants in dental public health programmes have an impact on the economics of sealants, as well as inefficient and inconsistent caries reduction, as many times people are not available for follow-ups. Fissure sealant application also takes a great deal of dental health providers' time. Therefore, the sealants of choice for dental public health programmes must not only be the best but must also be appropriate, i.e. the materials must not only exhibit excellent retentive properties, but also must be operator friendly and amenable to field settings. In this context, a resin-based sealant that can work under conditions of slightly compromised moisture control (as in field settings) that can also obturate the pits and fissures maximally would hold a great promise to the success of community sealant programmes.

Therefore, the present study was conducted as a formative research with the null hypothesis that there was no difference in the penetrative and adaptive properties of moisture tolerant resin sealant and conventional resin sealant *in vitro*. The moisture-tolerant sealant could then be tested further in field settings for effectiveness. The *in vitro* nature of the study facilitated inclusion of a parameter of interest, least reported in previous similar studies, namely the factor of fissure morphology. As this factor cannot be evaluated *in vivo*, the study was also conducted with the secondary aim to assess the need to perform fissurotomy procedures in a subsequent effectiveness study based on the differences (if any) in penetration and adaptation in various fissure morphological types.

Materials and method

Preparatory phase of the study

The protocol of the study was reviewed by the Ethical Committee, Sumandeep Vidyapeeth, Piparia, Vadodara (Gujarat, India) and the study was granted ethical clearance.

A pilot study was conducted with 18 teeth specimen allocated to receive one of the two sealants to be compared, in order to confirm the feasibility of methodology (described below) and to estimate sample size for the study.

Study design and sample size

The study was an *in vitro* comparative experimental trial. The teeth specimens were randomly allocated in equal numbers to receive either of the two sealants under investigation. The scanning electron microscopy (SEM) scientist was blinded to the study materials used and fissure type classification of the teeth specimen under investigation.

The sample size of the present study was estimated to be 50, based on the descriptive statistics obtained from the pilot study with 95% confidence interval (CI) and 80% power.

Collection of teeth specimen

The sample of extracted teeth comprised of sound morphologically intact premolars and third molars extracted for orthodontic reasons and disimpaction respectively.

They were collected from the Department of Oral and Maxillofacial Surgery, KM Shah Dental College and Hospital, Piparia, Vadodara, India and private dental clinics in Vadodara. Teeth with caries, abrasion, attrition, erosion, fluorosis and developmental anomalies were excluded.

Pre-treatment procedures

Storage and disinfection

The extracted teeth were cleaned under running tap water to remove any visible blood or tissues and stored in 0.9% saline. Prior to handling the teeth specimen for the experiment purpose, the teeth specimen were autoclaved through a 40-minute cycle at 121°C and 15 psi pressure as per the Centre for Disease Control and Prevention (CDC) guidelines[11].

Cleaning

Surface cleaning of pits and fissures was done to remove plaque and pellicle so that optimal etching of the enamel could be obtained. The occlusal surfaces of teeth specimen were cleaned with ultrasonic scaling unit (ART-P6 Pro Unicorn Denmart; 220 Vac ± 5%, 50-60Hz 28VA; Working frequency: 26KHz-32 KHz) and with a bristle brush using pumice and water slurry, free of oil and fluoride as fluoride and oil may hinder with resin bonding. After cleaning the pits and fissures, copious amounts of water were sprayed to remove any residual pumice from the pits and fissures.

Mounting of teeth specimen

The cleaned teeth were embedded in plastic blocks using cold-cure resin (Quick Ashwin, Rapid Repair, Dr Jagdishlal Sethi), exposing the crown part of the tooth and 2 mm of root below the cementoenamel junction to facilitate the sectioning of crown from the root. The mounting helped in easy handling of the specimens during sealant application procedures and, to an extent, standardised the treatment procedures and conditions. After mounting, the deepest part of the fissure was identified by probing with a No. 3 explorer and was marked using an indelible marker pen for identification on the buccal surface along the corresponding line of section.

Sealant materials used in the study

The sealant materials used in the study were Seal-Rite (Pulpdent Corporation) and Embrace Wet Bond (Pulpdent Corporation).

Seal-Rite is a conventional resin-based pit and fissure sealant. It is 34.4% filled. Embrace WetBond is a moisture tolerant resin-based sealant. It is a light cured material. It is a wet-bonding sealant. Owing to its hydrophilic property, it bonds chemically to the tooth and does not require a dry field. It is 36.6% filled. Both the fluorides are light curing, fluoride releasing and radiopaque.

Sealant application procedure

The procedures followed in the sealant application were in accordance to the manufacturer's instructions. The specimens were dried thoroughly with uncontaminated, oil-free and moisture-free compressed air, 38% phosphoric acid etch gel (Etch-Rite, Pulpdent Corporation) was applied to the treatment site for 20 s and then rinsed with copious amounts of water through a three-way water syringe. Etched surfaces were dried with clean, uncontaminated compressed air and were checked for a frosty or chalky white appearance. An applicator tip on a sealant syringe was placed on one end of the treatment site and the sealant was carefully allowed to flow. The sealant was applied to the pits and fissures. The material flowed from cusp to cusp, but did not cover the marginal ridges. The sealant was then light cured for at least 30 s using a light curing unit (Ultra-lite 500 EW, Unicorn Denmart).

The above-mentioned procedure was common to both the sealants except that for Embrace WetBond, prior to sealant application, the etched surfaces were moistened with a moist tissue wipe as the material was hydrophilic and binds to a wet tooth surface.

Sectioning of teeth specimen

Twenty-four hours after the sealant application, the teeth specimen were sectioned buccolingually using a slow-speed micromotor at 6,000 rpm (Marathon N1 SDE-H37L/SMT Saeyang Microtech; 1,600–40,000 rpm/minute) using a diamond disc (Dumont Instruments; 0.3 mm) at the deepest part of the fissure under continuous water spray. Another parallel sectioning made at a distance of 1.5 mm from the former yielded a tooth section 1.5 mm in width. The crown was then separated from the root. The teeth specimens were stored in distilled water until they were transported for SEM analysis to prevent specimen dehydration and shrinkage of sealant.

SEM

The specimens were examined by a scientist in the SEM Laboratory, Indian Institute of Science, Bangalore, with SEM (JEOL JSM-450 A). The same scientist did

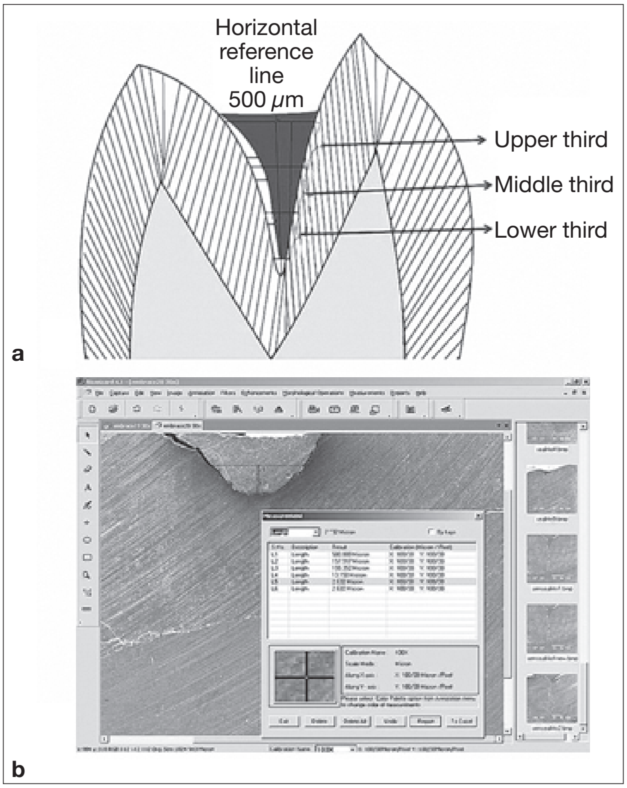

Fig 1 Measurements of penetration and adaptation **(a)** Schematic diagram **(b)** Using De Winter Biowizard version 4.1 image analyser software.

the SEM analysis for all of the specimens. The specimens were dehydrated for 24 h, which was a prerequisite for specimen mounting. The process of dehydration involved replacement of water by organic solvents and subsequent removal of the organic fluids by drying. After drying, the specimens were subjected to gold sputtering. The sputter coating removed or reduced the electric charge of the specimens, as the scanning electron microscope is sensitive to specimen charge. For this, the specimens were mounted on aluminium stubs using double-sided adhesive tape; they were mounted in such a way that the area to be studied faced upwards. The mounted surfaces were then coated with a thin layer of pure gold using an ion sputtering unit. The stubs were then placed in the vacuum chamber of the scanning electron microscope. The accelerating voltage, angle of tilt, and the aperture were adjusted to suit the specimen to optimize the quality of the micrograph. The specimens were viewed under 30×magnification under the scanning electron microscope and photomicrographs were generated for image analysis.

Classification of fissure forms

In the present study, fissure configurations were categorized according to Nagano's classification of fissure types. Nagano classified fissure forms into five types as when viewed in cross-section:

- V type: ample in top and gradually narrowing to the base.
- U type: almost the same width from top to base.
- I type: a very narrow groove.
- IK type: a very narrow groove associated with a large space in the base.
- Inverted Y type: inverted funnel shape with a narrow groove.

These fissure types have been correlated with fissure depth; for instance, the V type with a superficial, shallow depth, the U type with an average depth and other types associated with marked depth[1]. In the present study, a picture guide was used to classify the fissure forms.

Image analysis

The SEM images were analysed using De Winter version 4.1 image processing software. It is a new generation image analysis software to perform analysis in the simplest way. SEM images that were in TIFF format were exported to Biowizard Image Analysis software. Measurement tools in the software were used for penetration and adaptation of fissure sealants as described below.

Measurements

In the present study, measurements were performed using the methodology recommended by Covey et al[12]. First, a horizontal reference line (L_1) of 500 μm was constructed between cusp slopes at 30×magnification of the SEM image (Fig 1).

Perpendicular to this line, another line (L_2) was constructed from the reference horizontal line to the bottom of the fissure. The distance between L_1 and the bottom of the fissure was measured and it was recorded as the fissure depth. The penetration depth of the sealant was denoted by line L_3, which was the distance between L_1 and the lowest point (nearest to the fissure base) where the sealant could be detected. The mean of the greatest widths of the gaps observed between the fissure wall and the sealant at the upper third, middle third and

Table 1 Penetration and adaptation of sealants according to fissure form

Fissure form	Mean fissure depth (µm) Mean ± SD	Mean percentage penetration Mean ± SD	Mean width of widest gap between tooth and fissure sealant in upper third of penetrated portion (µm) Mean ± SD	Mean width of widest gap between tooth and fissure sealant in middle third of penetrated portion (µm) Mean ± SD	Mean width of widest gap between tooth and fissure sealant in lower third of penetrated portion (µm) Mean ± SD	Mean width of widest gap between tooth and fissure sealant in the entire penetrated portion (µm) Mean ± SD
U	385.69 ± 152.69	97.78 ± 3.07	7.83 ± 10.74	5.70 ± 5.78	13.39 ± 18.91	8.98 ± 7.85
V	232.46 ± 81.18	92.82 ± 10.71	4.63 ± 6.86	2.27 ± 3.99	9.21 ± 13.34	5.36 ± 5.15
I	889.60 ± 241.56	88.57 ± 18.16	3.87 ± 5.30	7.18 ± 8.69	13.22 ± 12.58	8.09 ± 5.77
IK	987.02 ± 170.03	78.12 ± 29.85	22.17 ± 21.03	19.20 ± 4.98	16.79 ± 8.02	19.39 ± 9.07

lower third of fissures penetrated by the sealant was recorded as the adaptation value in micrometers.

Statistical analysis

The recorded data were compiled and entered in a spreadsheet computer programme (Microsoft Excel 2007) and then exported to data editor page of SPSS version 11.5. Dependent variables to be studied were fissure depth (in μm), penetration of sealant (in μm), greatest width of gap between fissure wall and sealant in upper third, middle third and lower third of the sealed portion (in μm). Independent variables were fissure form and sealant materials, of which fissure form was a variable that could not be controlled.

Descriptive statistics included computation of percentages, means and standard deviations. Inferential statistics was performed for testing the hypotheses. A Chi-square test was performed to find out if the number of specimens with various fissure types allocated randomly to receive either of the two sealants differed in a statistically significant manner. A one-way ANOVA test was carried out to compare the mean percentage penetration between different fissure forms. As the distribution for the mean width of gap between the fissure wall and sealant (measure of adaptation) did not follow a normal distribution, a Kruskal Wallis test was performed to compare fissure types with respect to the measure of adaptation. Dunn's post-hoc test (non-parametric) was applied suitably to determine the contributors of significance. Dunn's post-hoc test was performed using a GraphPad Prism statistical programmer. Independent samples *t*-test was performed to compare the mean percentage penetrations between the sealant and sealant groups. The Mann-Whitney U test, the non-parametric counterpart of independent samples *t*-test was used to compare the measure of adaptation in both the sealant groups. A two-way ANOVA test was applied to test if there was any effect of interaction between the sealant material used and the type of fissure on the outcome variables. For all the tests, confidence intervals and P-values were set at 95% and 0.05, respectively.

Results

The distribution of sample specimen according to fissure form and sealant received revealed that the number of observed fissure forms in the two sealant groups were not found to differ significantly ($\chi^2 = 0.513$, $P = 0.916$). The inverted Y fissure form was not observed in any of the specimens in the study. Frequency of I form was the highest (36%), followed by U form (32%), V form (22%) and IK form (10%).

Penetration and adaptation values of fissure sealants according to the fissure form are presented in Table 1. IK fissures were the deepest fissures, with a mean fissure depth of 987.02 ± 170.03 μm and most shallow fissures were the V fissures, which had a mean fissure depth of 232.46 ± 81.18 μm. The mean percentage penetration of sealants was found to be the highest for the U fissure form (97.78 ± 3.07) and poorest mean percentage penetration was observed in the IK form (78.12±29.85). In U and V fissure forms, the mean width of the widest gap between tooth and fissure sealant was greatest in the lower thirds, followed by the upper third and middle third of the sealant-penetrated portion of the fissures. In the I form, the greatest mean width of gap was observed in the lower third, followed by the middle third and lower third of the sealant-penetrated portion of the sealant. In the IK form, the least mean width of gap between sealant and fissure wall was found in the lower third and the greatest gap width was found in the upper third of the sealant-penetrated portion of fissure. It was found that in the entirety of the sealant penetrated portion of fissures, the mean greatest gap width between the sealant and fissure form was observed for the IK form (19.39 ± 9.07 μm) and the least value was recorded for the V form (5.36 ± 5.15 μm).

One-way ANOVA revealed no statistically significant differences in penetration between the different fissure foms (F = 2.491, P = 0.072). The Kruskal Wallis test was applied to study the relationship between fissure type and adaptation as the numeric data for adaptation did not follow normal distribution. From Table 2, it can be noted that the mean rank (mean width of gap between the fissure wall and sealant in the entirety of the sealant-penetrated portion) was highest (indicating poor adaptation) for the IK type (41.20), followed by the U type (26.16), I type (25.31) and V type (17.73) and the differences were found to be statistically significant (Kruskal Wallis test statistic = 8.978, P = 0.030). Dunn's post-hoc test demonstrated that the differences in mean rank were attributed to the differences in adaptation in V and IK ($P < 0.05$), which chiefly contributed to the statistical significance.

Higher mean percentage penetration was observed with Embrace WetBond (95.19 ± 10.32) compared to Seal-Rite fissure sealant (87.62 ± 19.24). Examining the mean width of gap between the fissure wall and sealant, it can be seen that both the sealants adapted relatively poorly in the lower third of the sealant-penetrated portions of fissures. Seal-Rite exhibited apparently better adaptation in the upper third as compared to the middle third of the sealant penetrated portion, while Embrace WetBond adapted better in the middle third as compared to the upper third portion. The mean width of gap between the fissure wall and sealant in the entirety of penetrated portion of fissures was slightly lesser for embrace (8.86 ± 6.38) compared to Seal-Rite (8.95 ± 8.68) (Table 3).

The differences in penetration of Seal-Rite and Embrace WetBond were not found to be statistically significant (t = -1.736, P = 0.089). Also, the mean ranks (adaptation) were not found to differ significantly for the two sealant materials (Mann Whitney U test statistic = 289.500, P = 0.655).

In order to test for the source of variation and interaction between fissure form and sealant material with respect to the measure of adaptation in case of non-parametric data, numeric data for adaptation was log transformed for normality. Two-way ANOVA tests were performed on the normalized data and the results of the same are presented in Table 4. From the table, it can be inferred that sealant type and interaction did not account significantly for the differences in adaptation (F = 0.01578, P = 0.9006 and F = 0.2440, P = 0.8651, respectively). However, fissure morphology was found to contribute significantly to the differences in adaptation among the various fissure forms (F = 4.681, P = 0.0066). This implies that if fissure form had no effect overall, there was only a 0.66% chance of randomly observing an effect as big (or bigger) in an experiment of this size and the effect is considered very significant.

Discussion

The present study explored the effect of fissure morphology on the penetration and adaptation of a conventional resin-based sealant and a moisture tolerant resin-based sealant *in vitro* by SEM. Extracted premolars and third molars were included in the study sample as they were the most commonly extracted sound posterior teeth (for orthodontic reasons and disimpactions respectively). As they were the teeth that have remained in the oral cavity for the least amount of time, they probably provided the best possible standardised conducive environment for the application of sealant on the basis of histology of fissures with regard to cellular elements of enamel organ in the fissure and enamel porosities[13].

In the present study, sectioning was performed under a continuous jet of water to reduce heat generation. As only one section from the deepest part of the fissure was required in the present study, the use of slow-speed micro-motor driven diamond disc is justified. All the sectioned specimens were stored in distilled water until they were transported for SEM analysis. This was done to keep the specimen hydrated in order to minimize resin shrinkage. It has been reported that oral environment simulation with artificial saliva does not influence penetration of sealants[14] and therefore, distilled water storage was considered adequate for hydration.

SEM is to date the most precise instrument to study hard tissues. In addition to routine imaging at a 100 times greater resolution than optical microscopes, and with a focal depth over 10 times greater, SEM allows for detailed viewing and measurements. In the present study, fissure types were classified according to how they appeared in the SEM images with the aid of a standard picture guide.

Table 2 Relationship between fissure form and adaptation

Fissure form	N	Mean rank
U	16	26.16
V	11	17.73
I	18	25.31
IK	5	41.20
Total	50	

Measure of adaptation
(mean width of gap between fissure wall and sealant in the entire portion of fissure penetrated by the sealant)

Kruskal Wallis test statistic = 8.978
Df = 3
P = 0.030(S)

Dunn's Post-hoc test

Comparison	Mean Rank Difference	P- Value
U vs V	8.429	P > 0.05 (NS)
U vs I	0.8507	P > 0.05 (NS)
U vs IK	-15.044	P > 0.05 (NS)
V vs I	-7.578	P > 0.05 (NS)
V vs IK	-23.473	P < 0.05 (S)
I vs IK	-15.894	P > 0.05 (NS)

Table 3 Penetration and adaptation of the two sealants

Fissure Sealant	Mean fissure depth (μm) mean ± SD	Mean percentage penetration mean ± SD	Mean width of widest gap between tooth and fissure sealant in upper third of penetrated portion (μm) mean ± SD	Mean width of widest gap between tooth and fissure sealant in middle third of penetrated portion (μm) mean ± SD	Mean width of widest gap between tooth and fissure sealant in lower third of penetrated portion (μm) mean ± SD	Mean width of widest gap between tooth and fissure sealant in the entire penetrated portion (μm) mean ± SD
Seal-Rite	612.09 ± 351.91	87.62 ± 19.24	5.67 ± 10.67	6.28 ± 8.88	14.91 ± 16.97	8.95 ± 8.68
Embrace WetBond	574.95 ± 354.21	95.19 ± 10.32	8.60 ± 11.19	7.39 ± 6.92	10.58 ± 11.50	8.86 ± 6.38

Table 4 Two-way ANOVA table for simultaneous effect of fissure form and sealant type on adaptation (after log transformation of data for normality)

Source of variation	Percentage of total variation	Sum of squares	Mean square	F	df	P value
Sealant (S)	0.03	0.7695	0.7695	0.01578	1	0.9006 (NS)
Fissure Form (F)	24.59	684.8	228.3	4.681	3	0.0066 (S)
Interaction (S x F)	1.28	35.69	11.90	0.2440	3	0.8651 (NS)
Residual		204.8	48.76		42	

In the present study, highest mean fissure depth values were observed among IK and I forms; U forms had medium fissure depths and V forms were shallow fissures. The findings were in concordance with the description of Nagano[1].

The mean percentage penetration was highest for the U forms. Tagtekin et al[15] have also reported greater penetration in medium fissures compared to shallow and deep fissures. In a study conducted by Durmusoglu et al[16], a higher number of U fissures and medium depth fissures were found to exhibit complete penetration by the sealant. Grewal and Chopra[4] reported greater penetration of sealants into V forms as compared to U forms; the reason why shallow fissures were not penetrated completely in the present study as compared to medium forms might be due to some air trapped at the bottom of shallow fissures. The poorest mean percentage penetration was observed in the present study among the specimens with the IK form followed by the I form, and the finding was in affirmation with results put forth by Grewal and Chopra[4]. Powell and Craig[17] have observed in their study that narrow, deep and constricted fissures were penetrated poorly and wide fissures were more often completely penetrated. Petrović et al[18] also reported poorer penetration of sealants in deep fissures compared to shallow fissures. The poor penetration in deep, constricted fissures can be explained by comparing the fissure crevices to capillary spaces. Capillary penetration follows Poiesuille's equation $dx/dt = (\gamma \cos\theta/2\eta)_t\, r/2x$, where dx/dt is rate of capillary penetration, γ is the surface tension of the liquid, η is the viscosity of the liquid, θ is the contact angle of the liquid on capillary wall, r is the radius of the capillary and x is the length of liquid column at time t[19]. This indicates that the rate of capillary penetration decreases with decreasing capillary radius. The dimensions of narrow, deep fissures are smaller than the capillary radii used in Poisuilli's experiment and the rate of sealant penetration in them would be considerably slower. In spite of numerical differences in penetration values among the various fissure forms that were noted in the present study as described above, the differences were not found to be statistically significant. However, statistically significant differences between the fissure forms and/or depths have been reported by Grewal and Chopra[4]. This might be probably due to the fact that the deep I and IK fissures in the present study (88.57 ± 18.16 and 78.12 ± 29.85 respectively) were penetrated deeper compared to those in the study of Grewal and Chopra[4] (62.30% and 52.42% respectively). The better penetration of sealants in the I and IK forms in the present study may be attributed to the surface prophylaxis carried out, which probably permitted better etching and resin filling; however, specimen prophylaxis with a prophylactic agent was not performed in the study conducted by Grewal and Chopra[4]. Although the studies conducted by Duangthip et al[20], Petrović et al[18], Marks et al[21] suggest that fissure morphology has statistically significant effects on penetration ability, the results of those studies cannot be justly compared with the present study, owing to different fissure classification systems employed and different criteria used to measure penetration.

Adaptation was observed to be poorest for the IK form and best for the V form in the entirety of the sealant-penetrated portion of the fissure in the present study. This can be attributed to better etching patterns and good resin-bonding that could be achieved in the shallow, wide V form compared to the deep and constricted IK form. In the IK form, the mean value width of the widest gap between the fissure wall and the sealant was found to be the greatest at the upper thirds of the area of fissure penetrated by the sealant. This can be accounted to the presence of a prismless layer of enamel in the fissure system, which is resistant to acid-etching. The areas that typically did not etch were the entrance of the fissure and the fissure walls[22]. Poor etch patterns means poor resin bonding. Resin-based sealants shrink as they polymerise, creating stress up to 7 MPa within the resin mass[23-25]. Immediate bond strengths of approximately 17 MPa may be necessary to resist the contraction stresses that occur during polymerisation to prevent debonding[24]. Adaptation was found to be best for V fissures, supporting the belief that the etchant wets the shallow broad fissure systems and fails to etch deeper regions of the narrow fissures. The difference between adaptation in the IK and V forms may be due to organic remnants in the IK type forms, which remained inaccessible to cleaning. Tagtekin et al[15] also reported that sealants failed to adapt well to deep fissures compared to shallow fissures. In the study conducted by Durmusoglu et al[16], U forms exhibited slightly greater adaptation compared to V forms; however both U and V forms exhibited tight connections to enamel compared to I forms. IK forms were not reported in their study; therefore, no valid comparisons could be made. Unlike the study of Grewal and Chopra[4], in the present study, fissure morphology was found to influence adaptation in a statistically significant manner and the difference in adaptation between V and IK forms were found to contribute to the significance. This inference has serious implications, as sealant success depends on marginal adaptation. A compromise in the adaptation allows the occurrence of microleakage, i.e passage of bacteria, fluids, molecules and ions through the tooth–material interface, which can prompt caries lesion progression underneath the sealant.

In the present study, the mean percentage penetration was found to be higher for Embrace WetBond, the moisture tolerant resin-based sealant compared to Seal-Rite, the conventional resin-based sealant. The penetration values observed for Embrace WetBond were comparable to the penetration values reported by Courson et al[26]; in their study, they had compared Embrace WetBond with Delton FS, a traditional light cured resin-based sealant, and the latter exhibited a mean percentage penetration comparable to the mean percentage penetration of the conventional resin-based sealant used in the present study. With respect to adaptation, Embrace WetBond was found to fare slightly better with the lower mean gap width between the fissure wall and sealant in the entirety of the sealant-penetrated portion of fissure compared to Seal-Rite. In the present study, the differences in the penetration and adaptation of the two sealant materials were not found to be statistically significant. The statistically similar behaviour of the tested sealant materials may be explained by the *in vitro* experimental setting of the present study where moisture control was established. The differences observed between the two materials may be considered clinically significant although statistically insignificant as in a clinical or community-based set-up, with conditions of compromised moisture control, a conventional resin sealant that is not moisture tolerant may be expected to perform still poorer. Besides, the lower standard deviation values for mean percentage penetration and measure of adaptation were noted in Embrace WetBond compared to Seal-Rite, which is suggestive of less technique sensitivity in using Embrace WetBond. This further supports the superiority of Embrace WetBond.

Simultaneous consideration of fissure form and sealant type on penetration were assessed in the present study and Embrace WetBond was found to better penetrate invariably into all fissure forms compared to Seal-Rite. However, when effects on adaptation were assessed under simultaneous consideration of fissure form and sealant type, Embrace WetBond adapted better than Seal-Rite only in the U fissure forms; in the rest of the fissures, Seal-Rite was found to adapt better. This is probably due to the effect of fissure form on adaptation, which was found to be statistically significant in the present study. The slightly lower viscosity of Seal-Rite (34.4% filled) compared to Embrace WetBond (36.6% filled) might have resulted in its better performance with respect to adaptation. The results of a study conducted by Durmusoglu[16] and Stavridakis et al[27] in which the low viscosity sealant materials exhibited better adaptation than their high viscosity counterparts also support the possible role of viscosity on adaptation.

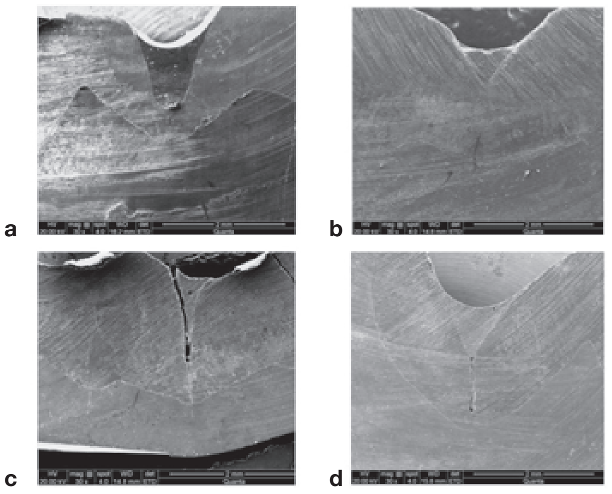

Fig 2 Scanning electron microscope image showing sealant within **(a)** U-shaped fissure **(b)** V-shaped fissure **(c)** I-shaped fissure **(d)** IK-shaped fissure.

Source of variation in adaptation and presence of any interaction between fissure form and sealant type operated with respect to the outcome variable of adaptation (as it was influenced by fissure form in a statistically significant manner) were explored in the present study. It was found that variation in fissure forms contributed significantly to the adaptation of sealant and variation due to sealant type and interactions were not found to be significant.

The results of the present study place fissure morphology as the paramount factor for adaptation and are in favour of the use of Embrace WetBond sealant, which holds a promise of performance in terms of penetration and adaptation. Thus, the findings of the study satisfactorily answered the research question whether there was any role of fissure morphology and performance of a moisture-tolerant resin sealant material with respect to penetration and adaptation into fissures or not. The standardisation measures employed in the various procedures involved in the study and the appropriateness of statistical methods used were the prime strengths of the study. However, at this juncture, a minor limitation of the present study needs to be mentioned as well. In the study, the teeth specimens were included in the sequence were collected till the sample size requirement was met, and therefore fissure form selection was not in the control of the investigator. Hence, in the study, subsample sizes in the various fissure types were different and equal distribution of fissure forms between the two sealant groups could not be achieved as well. Although most statistical techniques are amenable to deal with unequal sample sizes, balanced matrices increase the robustness of statistical techniques. However, this limitation cannot restrain the otherwise fool-proof methodology that allowed a thorough exploration of all factors related to the research question.

From the present study, it was concluded that although penetration varied across different fissure forms, fissure morphology was not found to affect penetration in a statistically significant manner. Hence, it may be inferred that to improve sealant penetration, fissure preparation using burs may not be necessary as fissure preparation with burs merely changes the morphology of I and IK forms into V or U forms. Further studies should be conducted to ascertain the effect of different techniques of air abrasion, ozone treatment etc on sealant adaptation, adhering to the philosophy of minimally invasive preventive dentistry. Improved fissure cleaning techniques must be developed in order to remove organic remnants from fissures completely, which would enhance the etching and resin-bonding.

Among the two sealants used in the study, Embrace WetBond sealant had greater mean percentage penetration. Embrace WetBond also exhibited slightly better adaptation than Seal-Rite. Advances in the sealant material technology through the development of low viscosity, moisture tolerant resin-based sealants, which can improve penetration as well as adaptation even into the deep and constricted fissure forms is, however, warranted.

Hence, in accordance to the conclusions drawn from the present study, it is recommended, especially for community/school-based preventive programmes, that a minimally invasive sealant application with a low viscosity moisture tolerant resin-based sealant be used.

References

1. Nagano T. Relation between the form of pit and fissure and the primary lesion of caries. Dent Abstr 1961;6:426.
2. Kitchens DH. The economics of pit and fissure sealants in preventive dentistry: A review. J Contemp Dent Pract 2005;6:95–103.
3. Aguilar FG, Drubi-Filho B, Casimiro LA, et al. Retention and penetration of a conventional resin-based sealant and a photochromatic flowable composite resin placed on occlusal pits and fissures. J Indian Soc Pedod Prev Dent 2007;25:169–173.
4. Grewal N, Chopra R. The effect of fissure morphology and eruption time on penetration and adaptation fissure sealants: An SEM study. J Ind Soc Pedod Prev Dent 2008;26:59–63.
5. Harris NO, Garcia-Godoy F. Primary Preventive Dentistry, ed 6. New Jersey: Pearson Prentice Hall; 2004:285–318.
6. Messer LB, Calache H, Morgan MV. The retention of pit and fissure sealants placed in primary school children by dental health services, Victoria. Aust Dent J 1997;42:233–239.
7. Bravo M, Garcia-Anilo I, Baca P, et al. A 48-month survival analysis comparing sealant (Delton) and fluoride varnish (Duraphat) in 6 to 8-year-old children. Community Dent Oral Epidemiol 1997;25: 247–250.
8. Poulsen S, Beiruti N, Sadat N. A comparison of retention and the effect on caries of fissure sealing with a glass-ionomer and resin-based sealant. Community Dent Oral Epidemiol 2001;29:298–301.
9. Simonson RJ. Glass ionomer as fissure sealant – a critical review. J Public Health Dent 1996;56:146–149.
10. Songpaisan Y, Bratthal D, Phantumvanit P, et al. Effects of glass ionomer cement, resin-based pit and fissure sealant and HF applications on occlusal caries in a developing country field trial. Community Dent Oral Epidemiol 1995;23:25–29.
11. Kohn WG, Collins AS, Cleveland JL, et al. Guidelines for infection control in dental health care settings – 2003. Centers for Disease Control and Prevention (CDC) MMWR Recomm Rep 2003;52:1–61.
12. Covey DA, Johnson WW, Hopper LR. Penetration of various pit and fissure sealants into occlusal grooves. IADR/AADR/CADR. 82nd General Session, 2004;3471. http ://iadr.confex.com/iadr/2004hawaii/techprogram/index.html. Accessed on 20 Dec 2009.
13. Galil KA, Gwinnett AJ. Histology of teeth in human unerupted teeth. J Dent Res 1975;54:960–964.
14. Aranda W, Courson F, Degrange M. In vitro evaluation of a simulated oral environment of different materials for pit and fissure sealing. European Cells and Materials 2005;9(Suppl1):73–74.
15. Tagtekin DA, Durmusoglu O, Ozyoney G, Sütçü C, Yanikoglu FC. Microscopic evaluation of different sealant materials. Balk J Stom 2009;13:99–104.
16. Durmusoglu O, Tagtekin DA, Ozyoney G, Bozzkurt FO, Yanikoglu FC. Light microscopy and SEM evaluation of three different fissure sealants. OHDMBSC 2005;4(4):26–32.
17. Powell KR, Craig GG. An in vitro investigation of penetrating efficacy of BIS-GMA resin pit and fissure coatings. J Dent Res1978;57: 691–695.
18. Petrović B, Marković D, Blagojević D. The impact of occlusal morphology on fissure sealant penetration. Serbian Dental J 2006;53:87–94.
19. Fan PL, Seluk LW, O'Brien WJ. Penetravity of sealants: I. J Dent Res1975;54:262–264.
20. Duangthip D, Lussi A. Effects of fissure cleaning methods, drying agents and fissure morphology on microleakage and penetration ability of sealants in vitro. Pediatr Dent 2003;25:527–533.
21. Marks D, Owens BM, Johnson WW. Effect of adhesive agent and fissure morphology on the in vitro microleakage and penetrability of pit and fissure sealants. Quintessence Int 2009;40:763–772.
22. Burrow MF, Burrow JF, Makinson OF. Pits and fissures: etch resistance in prismless enamel walls. Aust Dent J 2001;46:258–262.
23. Bowen RL, Nemoto K, Rapson JE. Adhesive bonding of various materials to hard tooth tissue: forces developing in composite materials during hardening. J Am Dent Assoc 1983;106:475–477.
24. Davidson CL, de Gee AJ, Feilzer A. The competition between the composite-dentin bond strength and the polymerization contraction stress. J Dent Res1984;63:1396–1399.
25. Davidson CL, Feilzer AJ. Polymerization shrinkage and polymerization shrinkage stress in polymer-based restoratives. J Dent1997;25:435–440.
26. Courson F, Legoff S, Aranda W. Penetration and marginal leakage of Embrace WetBond pit and fissure sealant: http://www.aluro.co.nz/supplies/pdf/Embrace%20WetBond_Penetration%20&%20Marginal%20Leakage.pdf.Accessed on 23 Aug 2010.
27. Stavridakis MM, Faves V, Campos EA, et al. Marginal integrity of pit and fissure sealants. Qualitative and quantitative evaluation of the marginal adaptation before and after in vitro thermal and mechanical stressing. Oper Dent 2003;28:403–414.