Comparative evaluation of marginal integrity of two new fissure sealants using invasive and non-invasive techniques: a SEM study

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In this era of preventive dentistry, many dental materials used for prevention of dental diseases are available. Since last few decades efforts are being directed towards prevention of dental caries which is one of the major dental diseases tormenting mankind. The main avenues available are plaque control, use of systemic and local fluorides and use of fissure sealants. There are many factors that contribute towards a successful sealant restoration such as properties of enamel, duration of etching, acid used for etching, manipulative variables. However, one of the prime factors governing the efficacy and life expectancy of a sealant is the marginal adaptability. This study was carried out on a total of 40 intact premolar teeth, which were divided in four main groups and subjected to evaluation under Scanning Electron Microscope. The parameters checked were the width and the number of marginal gaps. The results obtained showed that Teethmate had better marginal integrity and favored the use of invasive technique over non-invasive technique.

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INTRODUCTION

The anatomical pits and fissures of the teeth have long been recognized as susceptible areas for initiation of dental caries. Fissures or narrow isolated crevices and grooves that harbor food and microorganisms have been described as "the single most important anatomical feature leading to the development of occlusal caries." Though various preventive methods have been tried, accomplishments made towards prevention of occlusal caries are still unsatisfactory.

Robertson[7] reported that the caries potential was directly related to the shape and depth of the pits and fissures, and caries seldom began on smooth, easily cleaned surfaces.

Keeping in mind the proneness of occlusal surfaces towards caries, maintenance of oral hygiene in conjunction with fluoride therapy and prudent use of pit and fissure sealants seems to be the best preventive strategy.

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This would be most applicable in developing countries which are characterized by the predominance of the child and adolescent sectors of the population (nearly 40% of the total population) and with the lack of professional and auxiliary dental manpower, thus reducing the treatment possibilities available to the masses.

Lehner[6], stated that the concept of preventive programs for oral health should concentrate on the enhancement of natural protection, provision of added protection against specific disease, procedures to reduce the ability of microorganisms to produce disease, and the modification of behavior patterns to promote oral health and reduce disease.

Taking in consideration a developing country like India, the low dentist to population ratio makes the preventive measures towards oral health imperative. Even if the initial cost of preventive measures like sealants may be higher than the cost of restorative materials like dental amalgam, at long term basis sealants or any other preventive measure would be more cost effective as the tooth is being maintained in the state of health.

For variety of reasons fissure sealants have not been widely accepted by the dental profession, despite overwhelming evidence in support of caries prevention potential. The main problem usually encountered is towards microleakage leading to deterioration of the material and increased possibility of development of caries. A lot of work has also been done on invasive and non-invasive techniques used for the placement of sealants. However, not much work has been done on evaluation of these techniques in the manner they affect adaptability of sealants. Also, with introduction of new fissure sealant materials, it is pertinent to reexamine the physical characteristics and technique of fissure sealants.

Motivated by the role of sealants in caries prevention, this study was carried out to find out the most effective technique among invasive and non-invasive techniques and simultaneously evaluate two new sealants Teethmate F-1 and Fuji Ionomer III for marginal integrity under Scanning Electron Microscope.

MATERIALS AND METHODS

This study was carried out in the Department of Pedodontics and Preventive Dentistry, College of Dental Surgery, Manipal in collaboration with Indian Institute of Technology, Mumbai, India.

A total sample of 40 healthy, non carious, non hypoplastic, non fluorosed premolars freshly extracted for orthodontic purposes were collected. These 40 premolars were divided into four groups each containing 10 teeth.

Group 1 included teeth that were filled with Teethmate F-1 (Kurray Co. LTD, Japan) — Red using invasive technique. This group was further subdivided into two subgroups (containing 5 teeth each) according to the way it was sectioned for evaluation

Subgroup A: Occlusal surface topography.

Subgroup B: Bucco-Lingual Section.

Group 2 included teeth that were filled with Teethmate F-1 (Kurray Co. ,LTD. - Japan) Red using non-invasive technique. This group was also further subdivided into two subgroups (containing 5 teeth each) according to the way it was sectioned for evaluation

Subgroup C: Occlusal surface topography.

Subgroup D: Bucco-Lingual Section.

Group 3 included teeth that were filled with Fuji-lonomer III Light yellow(GC Corporation, Tokyo, Japan) using invasive technique. This group was also further subdivided into two subgroups (containing 5 teeth each) according to the way it was sectioned for evaluation

Subgroup E: Occlusal surface topography.

Subgroup F: Bucco-Lingual Section.

Group 4 included teeth that were filled with Fuji Ionomer III Light yellow (GC Corporation, Tokyo, Japan) using non-invasive technique.

This group was also further subdivided into two subgroups (containing 5 teeth each) according to the way it was sectioned for evaluation.

Subgroup 6: Occlusal surface topography.

Subgroup H: Bucco-Lingual Section.

All teeth samples were polished with pumice to remove any residual plaque or stains specially from the occlusal surfaces and stored in artificial saliva at room temperature. Artificial saliva was prepared by the College of Pharmaceutical Sciences, Manipal. Composition of Artificial Saliva was as given by Sieck *et al.*[8] in 1990.

For the teeth to be subjected to invasive technique (Group 1 and 3), fissures were prepared using contra angle stainless steel 1/4 round bur and a micromotor to ensure standardization and uniformity. For teeth subjected to non-invasive technique no preparation of the fissures was done.

Sealant placement was done using the material corresponding to that group and according to the instructions of the manufacturers.

Thermocycling was done to simulate the oral environment. All the groups were subjected to thermocycling at 5°C, 37°C and 55°C for 250 cycles with a dwell time of 30 seconds and stored for 15 days in artificial saliva before being tested for microleakage and bond strength.

The filled specimens were stored for 15 days in artificial saliva before being subjected to sectioning. In half of the teeth of groups 1 to 4, the root portions were cut and crown was trimmed from the sectioned side to achieve a sample thickness of 4mm as per requirements of the Scanning Electron Microscope (SEM Probe, Model no. SU-30, Cameca (France). No preparation was done on the occlusal surfaces (Subgroup A) which allowed to study the marginal topography of the fissure sealant and the other half was sectioned buccolingually (Subgroup B) which allowed to study the marginal gaps along the depth of the fissure. Sectioning was done using Arkansas stone and a diamond disc and care was taken to avoid damage to the filled sealants. The specimens were allowed to dry for 24 hours before subjecting them to gold sputtering. This was done to prevent any moisture contamination during SEM study.

The specimens were mounted on brass specimen stubs using silver paste, a vacuum resistant adhesive. The specimens were mounted in such a way that the area to be studied faced upwards. Each stub contained three to four specimens. The mounted surfaces were then coated with a thin layer (30 mm thickness) of pure gold using an ion sputtering unit (Hummer V,Technics, Springfield, Virginia) of 1000 volts for a period of 30 seconds.

This ensured proper conducting surface to the non-conducting specimens. These stubs were then loaded in a special tray, which could hold six brass stubs at one time. This tray, containing the specimen stubs, were later placed in the vacuum chamber of the Scanning Electron Microscope. The accelerating voltage, angle of tilt and the aperture was adjusted to suit the specimen to optimize the quality of micrograph. The surface was scanned and observed on the screen under different magnifications.

The topographic details, the number of gaps were noted in each group of samples, width of the marginal gaps was measured by placing the two pointers/indicator marks at the two extremes of the gaps and the distance between them as given by the computer was noted. The representative areas were photographed. The results were subjected to statistical analysis.

Table 1. Mean width (in micrometers) of gaps in groups.

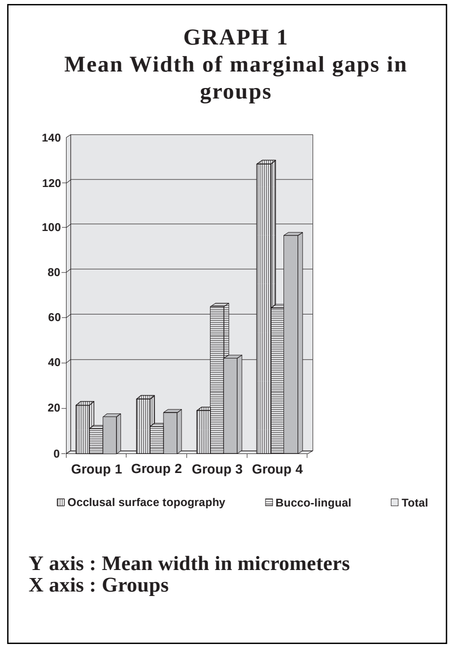
Group	Count	Mean	S.D.	SE.	95% Conf. mt. for mean
1	10	16.0990	8.9334	2.8250	9.77085 To 22.4895
2	10	18.0050	7.7004	2.4351	12.4965 To 23.5135
3	10	41.9485	26.5355	8.3912	22.9662 To 60.9308
4	10	96.4668	53.8728	17.0361	57.9285 To 135.0051
Total	40	43.1298	44.0836	6.9702	29.0312 To 57.2284

S.D.: Standard deviation
S.E.: Standard error, Conf. mt.: Confidence Interval

Table 2. Duncans Multiple Range test for width in groups

Mean value	Groups	Groups			
		1	2	3	4
16.0990	1				
18.0050	2				
41.9485	3				
96.4668	4	*	*	*	

* denotes pairs of groups significantly different at 0.050 level.



Graph 1. Mean width of marginal gaps in groups.

Table 3. Mean width (in micrometers) of gaps in subgroups.

Group	Count	Mean	S.D.	S.E.	95% Conf Int. for mean
A	5	21.3686	10.1408	4.5351	8.7773 To 33.9599
B	5	10.8294	2.7023	1.2085	7.4741 To 14.1847
C	5	24.0806	4.7843	2.1396	18.1402 To 30.0210
D	5	11.9294	4.2712	1.9102	6.6260 To 17.2328
E	5	19.0714	2.8779	1.2870	15.4981 To 22.6447
F	5	64.8256	16.3587	7.3158	44.5139 To 85.1373
G	5	128.7184	61.0622	27.3078	52.9009 To 204.5359
H	5	64.2152	14.1820	6.3424	46.6063 To 81.8241
Total	40	43.1298	44.0836	6.9702	29.0312 To 57.2284

S.D.: Standard deviation
S.E.: Standard error, Conf. ~ Confidence Interval

Table 4. Duncans multiple Range test for width in subgroups

Mean value	Sub Groups	Sub Groups							
		B	D	E	A	C	H	F	G
10.8294	B								
11.9294	D								
19.0714	E								
21.3686	A								
24.0806	C								
64.2152	H	*	*	*	*	*			
64.8256	F	*	*	*	*	*			
128.7184	G	*	*	*	*	*	*	*	

* denotes pairs of groups significantly different at 0.050 level.

RESULTS

This study was done on a total of 40 intact premolar teeth, which were divided in four main groups and subjected to evaluation under Scanning Electron Microscope. The parameters checked were the width and the number of marginal gaps.

Width of gaps

All the groups had 10 teeth each. Group 1 was subjected to invasive technique and filled with Teethmate F. This group showed the mean width of marginal gaps to be 16.09 ± 8.93 microns which was the lowest among all the groups. Group 2 was subjected to non-invasive technique, filled with Teethmate F and showed a mean width of marginal gaps to be 18.01 ± 7.70 microns. Group 3 was subjected to invasive technique, filled with Fuji Ionomer III and showed a mean width of 41.95 ± 26.54 microns. Group 4 was subjected to non-invasive technique, filled with Fuji lonomer III and showed a mean width of 96.47 ± 53.87 microns. (Table 1, Graph 1). On analysis, group 4 was found to be significantly different from the others. (Table 2).

On comparing the various subgroups, it was found that the minimum mean width of gaps was seen along the depth of sealants in case of Teethmate using invasive technique (Subgroup B), that is 10.83 ± 2.70 microns and the maximum mean width was seen along the margins of the sealant at the occlusal surface in case of Fuji III using non-invasive technique (Subgroup G), that is 128.72 ± 61.06 microns (Table 3, Graph 1). Analysis revealed subgroup G most statistically different from others followed by Subgroups H and F. (Table 4).

Table 5.Mean number of gaps in groups.

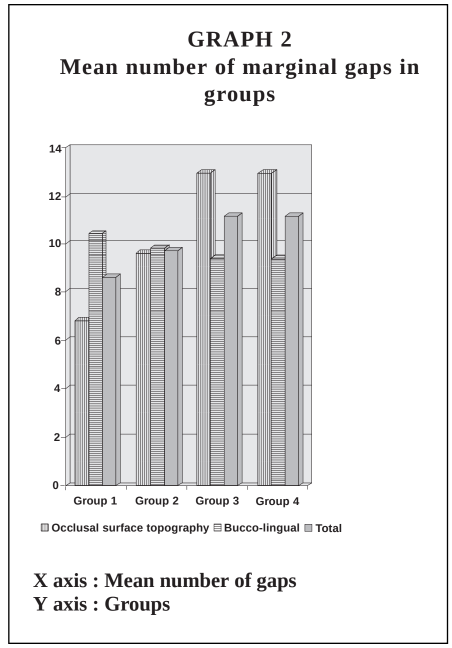
Group	Count	Mean	S.D.	S.E.	95% Conf Int. for mean
1	10	8.6000	2.6750	0.8459	6.6864 To 10.5136
2	10	9.7000	3.3015	1.0440	7.3382 To 12.0618
3	10	11.2000	2.5298	0.8000	9.3903 To 13.0097
4	10	11.2000	2.2509	0.7118	9.5898 To 12.8102
Total	40	10.1750	2.8365	0.4485	9.2679 To 11.0821

S.D.: Standard deviation
S.E.: Standard error Conf. mt.: Confidence Interval

Table 6. Duncans multiple Range test for number of gaps in groups.

Mean value	Groups	Groups			
		1	2	3	4
8.6	1				
9.7	2				
11.2	3	*			
11.2	4				

* denotes pairs of groups significantly different at 0.050 level.



Graph 2. Mean number of marginal gaps in groups.

Table 7. Mean number of gaps in subgroups

Group	Count	Mean	S.D.	S.E.	95% Conf Int. for mean
A	5	6.8000	2.1679	0.9695	4.1082 To 9.4918
B	5	10.4000	1.8166	0.8124	8.1444 To 12.6556
C	5	9.6000	2.4083	1 0770	6.6097 To 12.5903
D	5	9.8000	4.3243	1.9339	4.4307 To 15.1693
E	5	13.0000	2.0000	0.8944	10.5167 To 15.1693
F	5	9.4000	1.5166	0.6782	7.5170 To 11.2830
G	5	13.0000	1.4142	0.6382	11.2441 To 14.7559
H	5	9.4000	1.1402	0.5099	7.9843 To 10.0821
Total	40	10.1750	2.8365	0.4485	9.2679 To 11.0821

S.D. Standard deviation
S.E.: Standard error Conf. mt. Confidence Interval

Table 8. Duncans multiple Range test for number of gaps in sub-groups

Mean value	Sub Groups	Sub Groups							
		A	F	H	C	D	B	E	G
10.8294	A								
11.9294	F								
19.0714	H								
21.3686	C								
24.0806	D								
64.2152	B	*							
64.8256	E	*	*	*	*	*			
128.7184	G	*	*	*	*				

* denotes pairs of groups significantly different at 0.050 level.

Number of gaps

In the same samples along with the width of gaps the number of gaps were also noted.

Group 1 (Teethmate-Invasive) showed the minimum number of gaps, with the mean number of gaps being 8.6 ± 2.70; group 2 showed a mean number of 9.7 ± 3.30 and groups 3,4 showed the maximum number of gaps, with the mean number of gaps being 11.2 ± 2.53 for group 3 and 11.2 ± 2.25 for group 4 (Table 5, Graph 2). On analysis group 3 was found to significantly different. (Table 6).

On comparing the various subgroups, it was found that the minimum mean number of gaps was seen along the sealant interface at the occlusal surface of teeth in case of Teethmate using invasive technique being around 6.8 ± 2.17 (Subgroup A) and the maximum mean number was seen along the sealant interface at the occlusal surface in both invasive and non-invasive techniques of Fuji III being around 13.0 ± 2.0 (Subgroup E and G) (Table 7). On analysis subgroup E was most significantly different followed by subgroups G and B. (Table 8).

DISCUSSION

In this era of preventive dentistry, many dental materials used for prevention of dental diseases are available. Since the last few decades, efforts are being directed towards prevention of dental caries, which is one of the major dental diseases tormenting mankind. The main avenues available are plaque control, use of systemic and local fluorides and use of fissure sealants. Though use of fluorides have been shown to be very effective, the relative effect is seen to be least for the pits and fissures. The rationale of use of fissure sealant is that when it is applied on the caries prone fissures, it penetrates these pits and fissures and seals them from the oral environment. Also the microorganisms present in these fissures lose viability. The latest generation

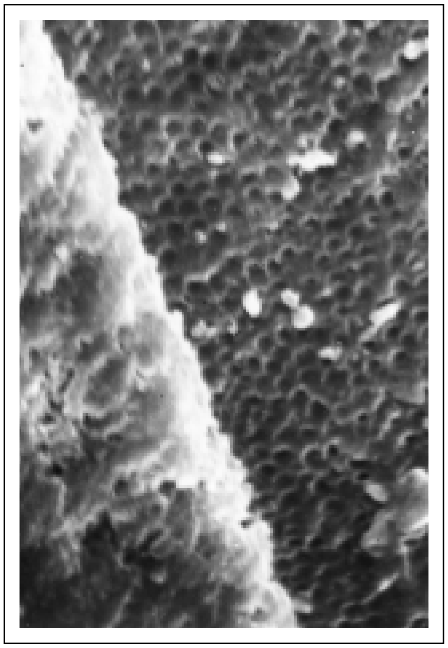


Figure 1. Sealant-tooth Interface using Teethmate F-1 with invasive technique at magnification of 5000 X.

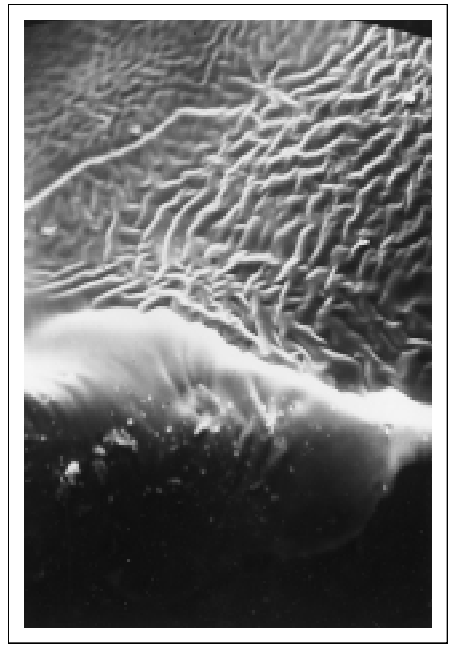


Figure 2. Sealant-tooth interface using Fuji-Ionomer III with Invasive technique at magnification of 5000 X.

sealants have the added advantage of having fluoride incorporated in them. Anticaries effect of fluorides is a well proven fact. There are many factors that contribute towards a successful sealant restoration such as properties of enamel, duration of etching, acid used for etching, manipulative variables. However, one of the prime factors governing the efficacy and life expectancy of a sealant is the marginal adaptability.

In the present study, a single investigator did the preparation of the samples and evaluation was undertaken with the help of a supervisor to reduce chances of error and remove any bias. As not much work has been done comparing invasive and non-invasive techniques, this study was designed to evaluate the effect of these techniques on marginal integrity and bond strength. For invasive technique 1/4 round bur was used for all teeth to ensure standardization in widening of fissures.

Teeth were stored in artificial saliva as given by Seick *et a1.*[8] to simulate the oral environment. Shortall,[10] gave a range of temperature from 0˚C to 68˚C for thermocycling. In the present study temperatures within this range, that is 5˚C, 37˚C and 55˚C were taken. Bullard, Lemnfelder and Russell[1] gave popular dwell time of exposure to each temperature extreme as 30 seconds, which was also followed in this study. Also, in the present study 250 cycles were carried out which was supported by Smith *et a1.*[11] who noted that there was no significant difference in microleakage between 250 and 500 cycles. Thermocycling was carried out to subject the materials and teeth to the extremes in temperatures seen in the oral cavity thus simulating the natural environment to make the study more authentic.

40 premolars which were studied for marginal adaptation under Scanning Electron Microscope were prepared as per requirements of SEM given by Goldstein and Yakowitz.[4] The occlusal surface of teeth in all the groups were evaluated so as to study the occlusal surface topography and gaps along the sealant-tooth interface and also evaluation was done for teeth sectioned in buccolingual way to study the same parameters along the depth of the sealant. The variables studied were the width and number of gaps present at the sealant enamel interface.

Crim and Shay[3] found under an SEM study that glass ionomer cavity liners showed marginal gap width considered too narrow to allow the free passage of bacteria. In the present study Fuji III was found to show greater gaps which would make it ineffective in preventing microleakage. This difference could be attributed to the different materials used and the difference in sample size, techniques and skills.

In the present study, no dye was used but the actual width and number of gaps were examined directly under SEN as it gives a more accurate picture and a direct visual observation of marginal adaptability of the sealant is possible which provides a more valid data directly related to microleakage in terms of possibility of passage of bacteria, saliva etc. Also, dye penetration may occur at the tooth restoration interface but marginal gaps may be too narrow to allow free passage to bacteria, indicating that dye penetration may not be a true indicator of bacterial or salivary contamination.[3]

Figure 3. Sealant-tooth interface using Teethmate F-1 with non-invasive technique at magnification of 30 X.

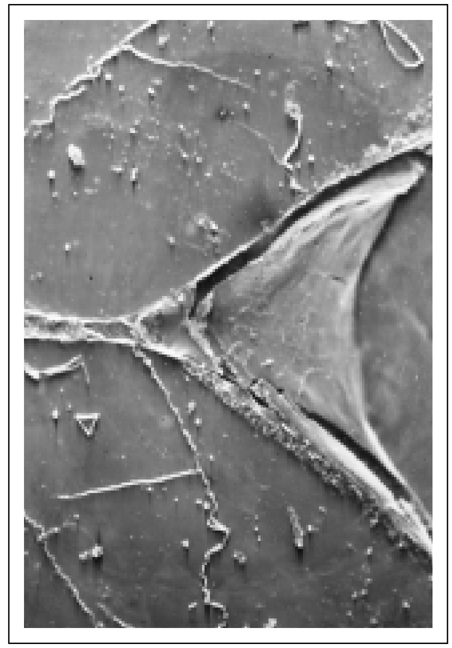


Figure 4. Sealant-tooth interface using Fuji-Ionomer III with non-invasive technique at magnification of 30 X.

Width of gaps

On analysis, it showed that the groups 1, 2 and 3 had little or no statistical difference but group 4 was highly significant and different from all the other groups (Table 2).

On comparison of subgroups for the width of gaps , it was noted that marginal integrity could not be maintained desirably using non-invasive method especially when Fuji III was used. The subgroups involving occlusal surface topography and bucco-lingual sections of Teethmate invasive and non-invasive and occlusal surface topography of Fuji III invasive were found similar with lesser width of gaps compared to other subgroups (Table 4). The better marginal integrity in invasive group could be attributed to better penetration of the sealant.

Le Bell,[5] gave good results when fissures were opened up before sealing, which allowed a plug of sealant to be formed, instead of thin layer of varying thickness and accounted for better adherence. This finding was substantiated in the present study.

Shapiro,[9] advocated mechanical preparation of sealants as the procedures widens and deepens the fissures by eliminating organic materials and a very thin layer of enamel resulting in a thicker layer of sealant and thus better adherence. In the present study the invasive group for both the materials was better in marginal adaptation than their respective groups.

Number of gaps

Groups 3 and 1 were significantly different (Table 6). Higher number of gaps seen in Fuji III again proved that the adaptability was not as good as with Teethmate.

On comparative evaluation of subgroups for number of gaps, the occlusal surface topography of Teethmate invasive or non-invasive and buccolingual sections of Teethmate non-invasive, Fuji III invasive or non-invasive had no significant difference in number of gaps; also buccolingual sections of Teethmate invasive or non-invasive and Fuji III invasive or non-invasive and occlusal surface topography of Teethmate non-invasive had similar number of gaps; and buccolingual sections of Teethmate invasive or non-invasive along with occlusal surface topography of Fuji III invasive or non-invasive had statistically similar number of gaps (Table 8). The overlap in the subgroups showed that number of gaps were not as distinctive feature in study of marginal adaptability as the width of the gaps. This may be substantiated by the fact even if a large number of gaps are present but their width is very less, they will not allow the passage of bacteria or their products, whereas large gaps even though less in number will allow the same.

It was noted that invasive technique using Teethmate F showed the best marginal adaptability among all the four groups followed by Teethmate F non-invasive group, Fuji Ionomer III invasive and Fuji Ionomer III non-invasive group in the same order. Similar findings were seen when different subgroups were considered, with the exception of occlusal surface topography of Fuji III-Invasive (subgroup E), which showed the mean width lesser than the occlusal surface topography of Teethmate, non-invasive (subgroup C - Table 4). Though apparently seen, this difference was not significant, and statistically the marginal adaptability along the occlusal surfaces was similar for the two groups. So, overall marginal adaptability was better for Teethmate, non invasive when compared to Fuji III-invasive. The better adaptability of Teethmate F could be attributed to the fact that Teethmate is formulated without filler, so it penetrates deeper into pits and fissures, thus sealing them completely. The poorer adaptability of Fuji III could be attributed to probable moisture contamination during setting, shrinkage or any difference in skills or technique. Invasive technique in both the sealants caused an improvement in adaptability, proving the importance of some sort of preparation before placement of sealants even if the properties of the material are not ideal. Craene, et al.[2] also recommended the use of invasive technique over non-invasive technique. As not much work has been done using the same parameters used in this study, proper comparative evaluation was not possible.

The importance of invasive technique was that it increased marginal adaptability of the sealants. In case of Fuji III it was seen that even though the property of the material was not good enough invasive technique increased adaptability significantly.

Therefore, invasive techniques can be considered for all types of sealant materials whereas non-invasive method should only be considered for materials with excellent properties.

CONCLUSIONS

The results obtained were subjected to statistical analysis and the conclusions drawn were as follows:

1. Teethmate F-1 has a better marginal adaptability to the fissures than Fuji-Ionomer III and shows lesser width and number of gaps at the sealant interface. This finding holds true for both the invasive and non-invasive techniques and the results were found to be statistically significant.
2. The invasive technique ameliorates the marginal integrity of the sealants as the difference found in the adaptability using the two techniques was statistically significant.

RECOMMENDATIONS

1. The invasive technique can be used for all types of materials but non-invasive techniques should be used only for materials with proven good adaptability.
2. It would be necessary to aid these findings by conducting a clinical study using these materials and parameters and further laboratory evaluation using a larger sample size, before drawing any definite conclusions.

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