Research Article

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Electron Microscopic Analysis of Three Different Resin Based Pit and Fissure Sealants at Sub Molecular Level: An In-Vitro Study

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ABSTRACT

Background: Pit & Fissure sealants application in high susceptibility tooth decay areas is one of the primary preventive measures to minimize the risk, reducing its incidence in pits and fissures, preventing the need for more invasive dental restorations.

Aim and Objective: This research was conducted in-vitro to evaluate qualitative and quantitative comparison of three different resin based pit and fissure sealants at sub-molecular level. The aim of this study was to evaluate in vitro the relationship between micro-leakage, resin infiltration and viscosity of three different resin based pit and fissure sealants.

Material Method: Thirty six freshly extracted premolars, for the purpose of Orthodontic treatment, were selected for the study. The teeth were then randomly divided into three groups of 12 samples each. Group- A consisted of Clinpro Sealant (3-M Unitech), Group B consisted of Helioseal F Sealant (Ivoclar Vivadent) and third Group- C consisted Fissurit Sealant (Voco). Teeth were thermocycled at a temperature range of 5-55 °C for 500 cycles, for 30 seconds. Each tooth was sectioned longitudinally & one half of every tooth section was utilized for micro-leakage testing and the other half for evaluating the length of resin tags.

Result: No statistical difference were found between Clinpro sealant vs Heliosealf Sealant and statistically significant ($P<0.05$) was observed between Clinpro Sealant vs Fissurit Sealant and Heliosealf Sealant vs Fissurit Sealant. No statistical differences were found between Clinpro Sealant vs Fissurit Sealant. Statistically significant difference ($p<0.05$) was seen between group Clinpro Sealant vs Heliosealf Sealant and Heliosealf sealant vs Fissurit sealant. The mean micro-leakage was lowest for Clinpro Sealant.

Conclusion: Clinpro Sealant (3-M Unitech) proved to be the best among the 3 test sealant groups in terms of its retention ability (based on the resin tag length and micro-leakage scores).

Keywords: Pit and Fissure Sealants, Microleakage, Electon Microscope, Sub- molecular.

INTRODUCTION

Dental health is often neglected by a vast majority of population. In the developing countries like India the prevalence of dental caries is very high particularly among the children and adolescents. A very extensive and comprehensive National Health Survey conducted in 2004 throughout India has shown dental caries prevalence as follows: 51.9% in 5-year-old children, 53.8% in 12-year-old children and 63.1% in 15-year-old teenagers. Currently Dental caries is a public health problem in India

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with a prevalence as high as 60-80% in Indian children a figure far more than asthma[1-2]. The American academy of Pediatric Dentistry has defined the term pit and fissure sealant a material that is introduced into the occlusal pits and fissures of caries-susceptible teeth, thus forming a micromechanically-bonded, protective layer cutting access of caries-producing bacteria from their source of nutrients[5].

Today, there is a wide spectrum of available sealing materials. A dental sealant is successful only if it firmly adheres to the enamel surface, and protects pits and fissures from the oral environment [12] The adherence of a sealant to the enamel surface can be assessed by measuring the length of resin tags it forms with the etched enamel surface and its ability to prevent any kind of microleakage. The ability of the resin to flow into the roughened enamel can be judged by its flow into the surface irregularities. The viscosity of any material determines its flow.

AIM

The aim of this study was to evaluate in vitro the relationship between micro-leakage, resin infiltration and viscosity of three different resin based pit and fissure sealants. This study was to establish a relationship between lengths of resin tags formed, viscosity and micro-leakage using three different resin based pit and fissure sealants.

OBJECTIVES

1. To correlate the length of resin tags with the occurrence of micro-leakage.
2. To evaluate the viscosity and thus the flow properties of fissure sealants.
3. To evaluate whether the viscosity of the fissure sealant has effect on the length of the resin tags.

MATERIALS & METHODS

The study was conducted in the Department of Preventive and Paediatric Dentistry at Buddha Institute of Dental Sciences & Hospital, Patna, Bihar. The experiments were performed at Nishka Labs, Hyderabad.

Source of Data

36 freshly extracted premolars for the purpose of orthodontic treatment were selected for study.

Inclusion Criteria: Coronal portion of the teeth free of cracks, caries, resorptive defects, fractures

Exclusion Criteria: Teeth with the following were excluded from the study: Resorbed roots, cervical caries, severe coronal caries, cracks in roots, severe root curvatures, open apices.

STUDY DESIGN

The collected teeth were cleaned off the calculus, plaque and debris with an ultrasonic scaler[10] and were stored in thymol solution. Pretreatment of the occlusal surfaces was done by cleaning the teeth with pumice slurry.

The teeth were then randomly divided into three groups of 12 samples each:

1. Group A- Clinpro Sealant
2. Group B-Helioseal F Sealant
3. Group C- Fissurit Sealant

Occlusal surface was etched with an etchant gel (37% phosphoric gel; SWISSTECH) for 15 seconds, and the tooth was then flushed with water for 15 seconds again to remove all of the etchant. The tooth was then dried with oil-free compressed air thoroughly to achieve a complete frosty white appearance. The sealant was allowed to flow into the fissures, avoiding incorporation of air bubbles. The sealant was light cured for 20 seconds according to the manufacturer's instructions.

Procedure for micro-leakage testing:

The teeth were coated with double layer of nail varnish to prevent the leakage of the dye. The occlusal surface was excluded. A different colour nail varnish was used for each group for easy distinguishing. All the groups were then immersed in 1% methylene blue for 24 hours. The samples were removed and gently brushed to remove excess dye. All the three groups were then subjected to thermocycling at a temperature range of 5-55 °C for 500 cycles[11], with a dwell time of 30 seconds. Each tooth was sectioned longitudinally in a mesio- distal direction. One half of every tooth section was

utilized for microleakage testing and the other half for evaluating the length of resin tags.

The sections for microleakage evaluation were cleaned and examined under stereomicroscope for the dye penetration and were scored as follows.

- 0= no dye penetration.
- 1= dye penetration down the mesial or distal wall.
- 2= dye penetration down the mesial and distal walls.
- 3= dye penetration underneath the sealant and down the mesial or distal wall.
- 4= dye penetration all around the sealant.

Apart from the scoring criteria, linear measurement of the dye penetration was recorded for each sample in each group

Procedure to evaluate length of resin tags formed:

The other half of the tooth section was prepared for evaluation under scanning electron microscopy. The resin tag lengths[12] were then measured. The average of each photograph was calculated.

Preparation of the samples for viscosity measurement:

The viscosity was checked by diluting the sealant with methyl methacrylate monomer liquid. Each sample was individually placed in the Brook's field viscosity meter at room temperature and the viscosity was measured in cP (centipoise). The obtained measurements were then subjected to statistical analysis.

RESULT

The recorded value were represented as mean ± standard deviation and were statistically analyzed using one way analysis of variance (ANOVA), Kruskal-Wallis test for multiple group comparison, post hoc turkey's test and Dunn's Multiple Comparison Test for group wise comparison.

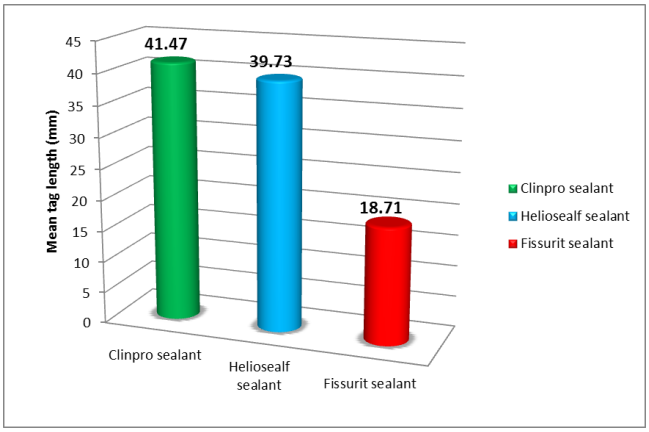
Resin tag length measurement:

Resin infiltration into the etched enamel surface was measured by the length of resin tags formed seen under a scanning electron microscope**.**

Table 1: Shows mean resin tag length for each group

Test Materials	Tag Length (Mean±SD)	P value
Clinpro sealant	41.47 ± 12.07	0.0007 (S)
Heliosealf sealant	39.73 ± 19.71	
Fissurit sealant	18.71 ± 9.957	

ANOVA test, P<0.05, significant (S), Non-significant (NS)



Graph 1. Comparison of Tag Length between Clinpro sealant, Helioseal f sealant and Fissurit sealant

Table 1 and graph 1 show the comparison of values obtained for tag length between the test material for Clinpro sealant is 41.47 ± 12.07; for Heliosealf sealant is 39.73 ± 19.7and for Fissurit sealant is 18.71 ± 9.957. No statistical difference was found between Clinpro sealant vs Heliosealf sealant. Statistically significant difference (p<0.05) was seen between group Clinpro sealant vs Fissurit sealant and Heliosealf sealant vs Fissurit sealant. The mean tag length was highest for Clinpro sealant.

Micro-leakage assessment:

Microleakage assessment was done dye penetration method under a stereomicroscope .It was measured linearly and by a scoring criteria.

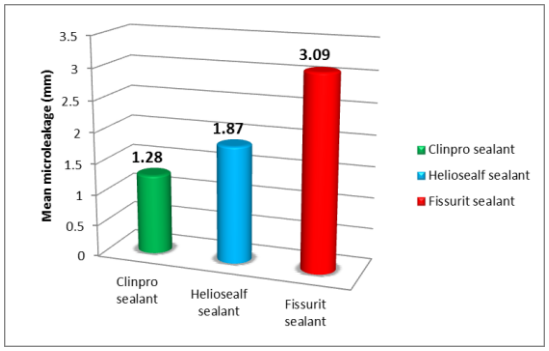
Results obtained for linear microleakage measurement

Table 2 shows the mean linear values of microleakge obtained

Test Materials	Microleakage (Mean±SD)	P value
Clinpro sealant	1.28 ± 0.68	<0.0001

Heliosealf sealant	1.87 ± 0.69	(S)
Fissurit sealant	3.09 ± 0.56	

ANOVA test, P<0.05, significant (S), Non-significant (NS)



Graph 2. Comparison of microleakage (mm) between Clinpro sealant, Heliosealf sealant and Fissurit sealant.

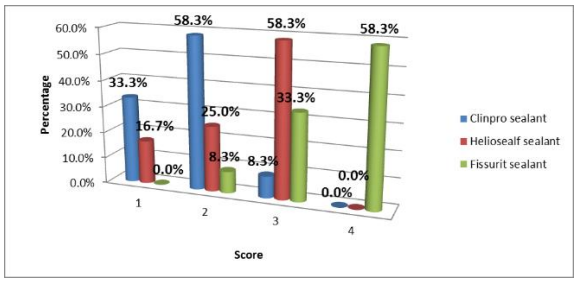
Table 2 and graph 2 shows the comparison of linear values (in mm) obtained for microleakage between the test materials for Clinpro sealant is 1.28 ± 0.68; for Heliosealf sealant is 1.87 ± 0.69 and for Fissurit sealant is 3.09 ± 0.56. Statistically significant difference (p<0.05) was seen between Clinpro sealant vs Fissurit sealant and Heliosealf sealant vs Fissurit sealant. The mean microleakage was lowest for Clinpro sealant.

Table 3: Results obtained for mean microleakage scores based on scoring criteria.

Groups	Number	Micro leakage				Mean Score	Median	P value
		0	1	2	3			
Clinpro sealant	12	4	7	1	0	0.75	1	<0.0001
Heliosealf sealant	12	2	3	7	0	1.42	2	
Fissurit sealant	12	0	1	4	7	2.5	3	

Kruskal-Wallis test, P<0.05, significant (S), Non-significant (NS)

Table 3: Shows the mean microleakage scores obtained based on scoring criteria



Graph 3: Mean microleakage scores and intergroup comparision of microleakage scores of of Clinpro sealant, Helioseal F sealant and Fissurit sealant

Table 3 and graph 3 shows the comparison of values obtained for microleakage based on the scoring criteria between the test material for Clinpro sealant, Helioseal F sealant and Fissurit sealant. The mean value of microleakage for Clinpro sealant is 0.75, Heliosealf sealant is 1.42 and Fissurit sealant is 2.5. No statistical differences were found between Clinpro sealant vs Fissurit sealant. Statistically significant difference (p<0.05) was seen between group Clinpro sealant vs Heliosealf sealant and Heliosealf sealant vs Fissurit sealant. The mean microleakage was lowest for Clinpro sealant.

Measurement of viscosity:

The viscosity of each test sealant was measured in a brooke's field viscometer .The process was repeated thrice for each sealant. The unit of measurement was centipoise.

Table 4 shows the individual viscosity of each sealant. Clinpro showed the lowest viscosity whereas Fissurit showed the highest viscosity.

Groups	Viscosity (cps)
Clinpro sealant	60 ± 0.53
Heliosealf sealant	74 ± 0.42
Fissurit sealant	90 ± 1.04

Tag length was maximum for Clinpro sealant followed by Helioseal F sealant and least by Fissurit sealant. Microleakage scores for Clinpro group were the least whereas Fissurit group showed maximum microleakage.

DISCUSSION

All the sealants used in this study are resin based sealants. Many studies have been conducted in the

past comparing resin based and glass ionomer based sealants, proving the former to have better retention and physical properties than the latter. Subramaniam et al evaluated the retention of glass ionomer used as a fissure sealant when compared to a self-cure resin-based sealant. At the end of the study period, the retention of the resin sealant was seen to be superior to that of the glass ionomer sealant[7]. Previous studies have been done comparing any of our test sealants (Fernandes et al, Sridhar et al, Motanari et al). It is for the first time that the three sealants (Clinpro ,Helioseal F and Fissurit sealants) have been tested for sealant penetration, microleakage and viscosity . Hence these sealants were chosen as our test materials

Table 1 and graph 1 show the comparison of values obtained for tag length between for Clinpro sealant is 41.47 ± 12.07; for Heliosealf sealant is 39.73 ± 19.7and for Fissurit sealant is 18.71 ± 9.957.Clinpro sealant showed the highest mean tag length whereas Fissurit showed the lowest mean tag length .Statistically significant difference ($p<0.05$) was seen between group Clinpro sealant vs Fissurit sealant and Heliosealf sealant vs Fissurit sealant. These differences can attributed to the individual viscosity and assosciated wettability of each test sealant. The lower the viscosity of the sealant, higher is the tag length. Mehrabkhani et al showed that the degree of penetration of a sealing agent into the interfacial micro-gap directly corresponds to the material's viscosity and potential to adequately wet restoration or tooth surfaces [13].Studies done by Prabhakar et al [9] and Motayam et al [13] also stands in agreement with our study. Marks et al stated that fissure morphology had sign ficant influence on microleakage, but did significantly impact penetrability [14].

Table 2 and Graph 2 shows and compares the mean value of linear measurement of miroleakage for each group in milllimetres. Clinpro showed the lowest mean measurement 1.28 ± 0.68; followed Heliosealf sealant (1.87 ± 0.69) and the highest values were obtained for Fissurit sealant (3.09 ± 0.56). Statistically significant difference ($p<0.05$) was seen between Clinpro sealant vs Fissurit sealant and Heliosealf sealant vs Fissurit sealant. This difference can be attributed to the variation in filler content of each sealant.

Clinpro sealant has the least filler (less than 6 %) content as compared to the two other sealants(Helioseal F -43% & Fissurit -55%) .

Table 3 and Graph 3 show the comparison of values obtained for microleakage scores obtained for the 3 sealants based on the scoring criteria . 58.3% of the 12 samples in Group A showed a microleakage score of 1 whereas 33% showed a score of 0. In Group B 58.3 % of the 12 samples showed a score of 2 and 25% showed a score 1. 58.3% in Group C showed a score of 3 and 33.3% had a score of 2. Therefore Clinpro sealant showed minimum amount of dye penetration and Fissurit sealant showed highest amount of dye penetration.

Sridhar et al attributed minimal microleakage obtained in Clinpro group as compared to the Helioseal F group, to the lesser filler loading of 6% in Clinpro fissure sealant when compared to filler loading as high as 43% in Helioseal F fissure sealant [8]. Table 4 shows Clinpro sealant showed least viscosity whereas fissurit sealant was the most viscous of all the 3 sealants. The variation in filler loading of each sealant can attributed to their difference in viscosities. According to Paris et al the penetrativity of a liquid into porous solids is described by its penetration coefficient (PC) which includes the characteristics viscosity, surface tension and contact angle of the liquid to the solid [15]. Kakaboura et al. reported that unfilled sealant penetrated deeper in narrow fissures than high viscosity resin composite and compomer [13]. Stavridakis et al showed that the low viscosity and unfilled sealant material (Teethmate F-1) exhibited better marginal adaptation than its high viscosity counterpart [16].

CONCLUSION

Clinpro sealant managed to be the best among the 3 test sealants in terms of its retention ability (based on the resin tag length and microleakage scores). Our study clearly indicated that there is a definite relationship between resin tag length,microleakage and viscosity of the material.Clinpro sealant clearly showed the longest resintag length with least microleakage alongwith the lowest viscosity which is responsible for its better retention. On the other hand Fissurit sealant showed the shortest resin tag length ,highest microleakage and the highest viscosity. These properties emerge to be the most

responsible quality for retention of the sealant. In this regard improving these qualities may enhance the cariostatic action and could be more effective to serve its role as an ideal sealant.

CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

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