Comparative evaluation of the length of resin tags, viscosity and microleakage of pit and fissure sealants – an in vitro scanning electron microscope study

A.R. Prabhakar, Sankriti A. Murthy, and S. Sugandhan

Abstract

Introduction

“Intellectuals solve problems; geniuses prevent them!!”

Caries occur five times more frequently in occlusal fissures, than on smooth surfaces.[1] Sealant not only prevents tooth decay before it starts, but also halts the progress. This effect relies on the sealant's ability to fill the fissure and not detach. Retention is provided by resin tags that form an effective mechanical interlock between resin material and enamel surface. Viscosity of sealant enhances penetration, thus helping in retention.[2] However it still remains to be evaluated as to how exactly do the factors, such as, resin tag length, microleakage and viscosity affect the clinical efficacy of these sealants. This study aims at evaluating the relationship between sealant viscosity, resin tag length and prevention of microleakage.

Materials and Methods

Three pit and fissure sealants were used in this study.

The following is the list of the materials and armamentarium used in this study.

Test Materials: [Figure 1]

Figure 1
Test materials used in the study, Helioseal, guardian seal and embrace wetbond
The collected teeth were cleaned off the calculus, plaque and debris with an ultrasonic scaler 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 10 samples each:

- Group E: Embrace wetbond sealant.
- Group H: Helioseal sealant.
- Group G: Guardian seal sealant.

Application of sealant

**Procedure for Helioseal and Guardian seal fissure sealants**

Occlusal surfaces of the teeth were etched with 37% phosphoric acid (Enamel preparator, Ivoclar) for 30 seconds and rinsed with water. The teeth were then dried with a mild oil-free air stream to achieve a characteristic frosty white, chalky appearance of enamel.[3] Two coats of bonding agent (G bond, GC) were applied (using applicator on the etched tooth surface) and light cured for 20 seconds using visible light curing unit (470 nm to 420 nm wavelength). Sealants were then applied and cured according to the manufacturer's instructions.

**Procedure for Embrace wet bond fissure sealant**

Occlusal surfaces of the teeth were etched with 37% phosphoric acid (Enamel preparator, Ivoclar) for 30 seconds and rinsed with water. With Embrace Wetbond, the typical dull, frosted appearance of the etched surface is not desired. Rather, the surface should be lightly dried and very slightly moist with a glossy appearance. To accomplish this, a cotton pellet was used to remove the excess moisture.[4] No bonding agent was applied. The sealant Embrace wet bond was applied as per manufacturer's instructions [Figure 2], followed by light curing for 40 seconds.[5] The prepared teeth were stored in double de-ionized distilled water.
Application of sealant Embrace wet bond as per the manufacturer's instructions in the study

**Procedure for microleakage 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.

- Red - Helioseal sealant
- Blue - Guardian sealant
- Orange - Embrace wetbond sealant

All the groups were then immersed inverted, (such a way that unpainted surface was in contact with the dye) 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, with a dwell time of 30 seconds. The procedure used to determine marginal leakage was similar to the one described by Theodoridou – Pahini and Tolidis. Each tooth was sectioned longitudinally in a mesio distal direction through the center of the sealant with a diamond wheel measuring 0.02 mm in thickness. The root portion of the teeth was then sectioned and removed. 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 by 2 independent examiners 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.

**Procedure for scanning electron microscopy and resin tag measurement**

The other half of the tooth section was prepared for evaluation under scanning electron microscopy.
The tooth sections were polished using a carbide stone. The polished sections were then decalcified using 37% phosphoric acid for 15 seconds to etch away any enamel mineral component not protected by sealants and then rinsed and stored in distilled water. The tooth sections were dried thoroughly under the heat lamp, and then mounted on brass rings using a non-conductor tape made of carbon. This was then applied to the sections, in the areas that did not need scanning. These mountings were then placed inside an ion sputtering device for 30 minutes using vacuum evaporation at 200 - 300 Å. The gold spluttered sections were then placed inside the scanning electron microscope of 20 kV capacity and photographs of the sections were obtained. The resin tag lengths were then measured [Figure 4]. The average of each photograph was calculated.

**Figure 4**
Scanning electron microscopy image showing the resin tag (t) and surface (s), and arrow pointing to the resin tag

**Preparation of the samples for viscosity measurement**

The viscosity was checked by diluting the sealant with methyl methacrylate monomer liquid. The viscosity of the monomer liquid was evaluated first. The liquid was placed in the sample holder of the Brooke's field viscometer and was calibrated at 60 revolutions per minute, 100% torque. Further, s-18 spindle was used and the reading was noted. 1 ml of the sealant was drawn out of its container and poured into a 10 ml test tube to which the methyl methacrylate liquid was added and mixed till the total liquid amounted to 10 ml (since the minimum amount needed to evaluate the viscosity was 10 ml). Each sample was individually placed in the Brook's field viscosity meter at room temperature and the viscosity was measured in cP (centipoise). Care was taken to see that the light exposure of the sealant was minimal. The obtained measurements were then subjected to statistical analysis.

**Results**

The recorded values were represented as mean ± standard deviation and range values, and were statistically analyzed using one way analysis of variance (ANOVA) for multiple group comparison, post hoc tukey's test for group wise comparison, the spearman's rank correlation coefficient for correlation analysis and the Mann Whitney test for multiple group comparison of the microleakage scores.

Table 1 and Graph 1 show the comparison of values obtained for microleakage between group E, group H and group G. The mean value of microleakage for group E is 0.4, group H is 1.0 and group G is 1.6. No statistically significant differences were found between the group E v/s group H and between group H v/s groups G.
Statistically significant difference ($p < 0.05$) was seen between group E v/s group G. Group E showed the least microleakage followed by group H and G.

**Table 1**
Mean microleakage scores and inter group comparison of microleakage scores of group E, group H, group G

**Graph 1**
Showing percentage of microleakage scores and inter group comparison of microleakage scores of the three groups in the study

**Table 2** and **Graph 2** show the comparison of values obtained for resin tag length between group E, group H and group G. The mean resin tag length for Group E is $10.14 \pm 4.84$; for group H is $9.65 \pm 4.28$ and for group G is $5.86 \pm 1.85$. The mean difference of group E v/s group H is 0.49, group G v/s group H is 3.79 and group E v/s group G is 4.28. No statistically significant differences were found between group E v/s group H and group H v/s group G. Statistically significant difference ($p < 0.05$) was seen between group E v/s G. The mean resin tag length was highest for group E.

**Table 2**
Mean difference of the resin tag lengths and inter group comparison of the resin tag length between group E, group H and group G

**Graph 2**
Showing the mean lengths of the resin tags and inter group comparison of the resin tag length of the three groups in the study

**Table 3** shows the relationship between the microleakage and resin tag length in group E, group H and group G. Statistically significant differences were seen in all the three groups. Group E showed a $p$-value of $P< -0.85$, group H showed a $P$-value of $P< -0.94$ and group G showed a $P$-value of $P< -0.96$. The relationship between microleakage and resin tag length was inversely proportional. As the resin tag length increased, the microleakage decreased.

**Table 3**
Relationship between resin tag length and microleakage of group E, group H and group G (correlation analysis)

**Table 4** and **Graph 3** show the compiled data showing the mean difference and range of resin tag length, microleakage scores and viscosity of group E, group H and group...
G. The viscosity of the group E was the lowest at 0.96 cP, group H was 1.59 cP and the group G was 1.92 cP.

Table 4
Compiled data showing the mean difference and range of resin tag length, microleakage scores and viscosity of group E, group H and group G

Graph 3
Compiled data showing the mean difference and range of the resin tag length, microleakage scores and the viscosity of the three groups in the study

Discussion

The occlusal surface, especially the pits and fissures of posterior teeth have been recognized for their high caries susceptibility over many years.[10] It is undoubtedly more vulnerable due to the unique morphology of the pits and fissures. Occlusal pits and fissures vary in shape, but are generally narrow (~ 0.1 mm wide) and tortuous, and are considered to be an ideal site for the retention of bacterial and food remnants. This is because the morphology renders the mechanical means of debridement inaccessible[11] as an average tooth brush bristle (0.2 mm) is too large to penetrate most of the fissures.[12]

Other factors such as lack of salivary access to the fissures, the close proximity of fissure base to the dentino-enamel junction and remnants of debris and pellicle in the fissures increase caries susceptibility of fissures by many folds. Therefore, to prevent initiation of caries in these fissures, the concept of pit and fissure sealants evolved.[13] The cariostatic properties of sealants are attributed to the physical obstruction of the pit and fissures. This prevents colonization of the pits and fissures with new bacteria and also prevents the penetration of fermentable carbohydrates to any bacteria remaining in the pits and fissures.[14] Various studies show strong correlation between the sealant and absence of caries.[15]

Requisites of an efficient sealant include: A viscosity allowing penetration into deep and narrow fissures even in maxillary teeth, adequate working time, rapid cure, good and prolonged adhesion to enamel, low sorption and solubility, resistance to wear, minimum irritation to tissues, and cariostatic action.[9] The third molars extracted for therapeutic purpose, which were free of caries, developmental defects, enamel microfractures and discoloration were included in this study, as previous studies[16,17] have revealed that any preexisting alteration of surface morphology of the tooth directly influenced the caries progression. As compared to the formaldehyde, thymol has no effect on the protein structure and neither does it alter the enamel structure. Hence in this study, 0.01% thymol solution was used as a storage solution and
disinfectant.[3] Some studies show that pumice prophylaxis does not completely and consistently remove the pellicle and debris, especially in the depth of the fissure.[18] Studies by Blackwood JA et al. showed that between enameloplasty, air abrasion and pumice prophylaxis, the least microleakage was seen with the conventional pumice prophylaxis.[19] In the present study, despite of all the controversies, conventional pumice method was used for cleaning prior to etching.

Timing of the sealant placement is critical. The teeth that have newly erupted are the ones that are most susceptible to caries and hence need protection from pit and fissures. But isolating them is the most difficult. Until now the only moisture tolerant sealants were glass ionomers.[20] Their mechanism of adhesion is ionic bonding, not micromechanical retention to an acid etched enamel surface. Paradi and co workers reported low sealant retention rates with glass ionomer cement (GIC).[21] Recently, resin-based sealant technology has introduced a moisture-tolerant chemistry. While traditional sealants are hydrophobic, Embrace™ Wetbond™ is hydrophilic. On light-curing, this sealant has physical properties similar to other commercially available sealants.[5] Embrace Wetbond incorporates di-, tri- and multifunctional acrylate monomers into an advanced acid-integrating chemistry that is activated by moisture. When placed in the presence of moisture, the sealant spreads over the enamel surface. Because of its unique chemistry, Embrace Wetbond is miscible with water and flows into moisture-containing etched enamel and combines with it.[5] Additionally, a longitudinal study done in vivo with embrace wetbond has shown a retention rate of 90%. Only 32 teeth out of the 334 teeth in the study required replacement of the sealant.[22]

Early in the development of sealants, it was recognized that the addition of fluoride to a sealant could have the potential benefits of additional caries protection. The fluoride release achieved high levels initially, with salivary fluoride concentration being high within 30 minutes after sealant placement; however, returned to baseline levels within 1-2 days after the sealant placement.[23] Evaluation of the combined use of fluoride and dental sealants has showed retention of 92% after 4 years. This suggests that pit and fissure sealants confer additional caries preventive benefits beyond those of fluoride therapy alone.[24]

A sealant is effective in preventing caries, only when it is retained in the fissures. Hence the retention becomes a major factor, influencing the efficacy of the sealant. The retention in resin based pit and fissure sealant is through micromechanical interlocking between the resin and the enamel. Mechanical retention of sealant is the direct result of resin penetration into the porous enamel forming tags. This occurs by capillary action. Monomer in the material polymerizes and the material becomes interlocked with the enamel surface.[25]

In the present study the mean length of tags obtained was in the range of 10 µm to 5µm.[19] Other studies by Gomez et al.[26] and Karina Tonini et al.[27] have also concurred with the measurements obtained. It was seen in our study that the pit and fissure sealant with the longer resin tags had lesser microleakage. Thus, it can be
postulated that, the longer resin tags formed, lesser will be microleakage. Other than surface tension, the viscosity of the sealant also influences the penetration of sealants. Viscosity is the resistance of a liquid to flow. This resistance of the fluid to flow is controlled by internal frictional forces within the liquid. A highly viscous fluid flows slowly. The viscosity is measured in units of MPa (Mega pascal) per second or cP (centipoise). Higher viscosity may cause poorer adaptation and incomplete penetration to the bottom of the pit and fissures resulting in decreased retention. With low viscosity sealants, there is a greater potential of the sealant to flow, spread more rapidly over the surface and penetrate. At times the size of the filler particles may be larger than the porosities of enamel. Faster penetration rates are found with larger holes, less dense liquids and those with high surface tension. On the contrary, the study by DM Barnes, P kihn, A Elsabach and JA von Fraunhofer has shown that the viscosity and flow properties of the fissure sealants do not affect their sealing ability. Hence, we tried to analyze the nature of relationship between viscosity and the other factors of pit and fissure sealants.

The viscosity of sealants can be assessed in centipoise units using ultrasonic vibratile viscometer or by conventional capillary tube method. The ultrasonic vibratile viscometer is a more accurate method as compared to the capillary tube method, as it gives a digital read out. The viscometer rotates the spindle in the liquid to overcome the viscous resistance to the induced movement, and thus a reading is obtained. In this study, the Brookfield viscometer was used. All Brookfield laboratory viscometers are accurate within the range of +/- 1%, and have a reproducibility within the range of +/- 0.2%, thus allowing the test results to be duplicated anywhere in the world when the same model instrument is used. In the present study, the viscometer utilized required a minimum volume of 10 ml sample. To achieve this, 1ml of each sealant was first diluted in 9 ml of the diluent, methylmethacrylate. Hence, the relative viscosities of the three sealants were obtained.

In the present study, the sealant with fluoride i.e. Guardian seal showed highest viscosity. It has been shown that sealant containing fluoride tends to be thicker than those without fluoride. The least viscous was the Embrace wetbond. Urethane monomer based sealant may confer more elasticity and adhesiveness to the resin than does Bisphenol A-Glycidyl Methacrylate (Bis-GMA). In this study, two of the sealants Helioseal and Guardian seal contained Bis-GMA, where as the Embrace wet bond contained di, tri and multiacrylate monomers. That may be the reason why the viscosity of Embrace wetbond was lower as compared to the other groups. Another factor thought to be central to the clinical success of sealants is microleakage. Microleakage is defined as the passage of bacteria and their toxins between restorative margins and the tooth preparation walls. Clinically microleakage is important when one considers that pulpal inflammation is more likely caused by bacteria than by chemical toxicity of restorative material. Microleakage may support the caries process beneath the sealant, hence the ability of the sealant to adequately seal the pits and fissures and prevent microleakage is important. If restoration is hermatically sealed, then the bacteria cannot survive.
Microleakage assessment may be qualitative or quantitative with different systems, including both simple and computer based methods. Dye penetration has been used in several studies, to assess the presence of marginal leakage around the sealant–enamel surface.[34,35] In the present study, a qualitative technique of dye penetration was used. Penetration of dye can indicate the lack of a perfect seal. The presence of a dye in the under penetration zone of the etched enamel can indicate a susceptible microleakage pathway. Clinically, this may imply that the remaining etched area could be a factor for development of caries by microleakage, if the sealant was partially or completely lost.

All the sealants used in this study showed some degree of microleakage and these factors agreed with previous studies.[7,31,36] The least microleakage was seen with the Embrace wetbond sealant, followed by Helioseal and Guardian seal. Complete penetration of sealant into complex fissure systems is difficult due to the phenomenon of closed end capillaries or isolated capillaries. Some lateral fissures arising from the main fissures also fail to be filled with sealant.[34] Hence, further research on the effect of fissure morphology on sealant microleakage and penetration are necessary. Stereomicroscope is the gold standard in microleakage studies and hence was used here. The SEM was used to measure the length of the resin tags as the SEM can produce very high-resolution images of a sample surface, and reveal details about less than 1 to 5 nm in size. Due to the very narrow electron beam, SEM micrographs have a large depth of field yielding a characteristic three-dimensional appearance useful for understanding the surface structure of a sample.[37]

It should be noted that the results of the present study are valid for in vitro conditions. Depending on the environment, all pit and fissure sealants may act differently due to other variables like type of fissures, preparation of fissures, enamel etching and conditioning, application of bonding agent and contamination of prepared surfaces of fissures. Appropriate method of application of sealants and viscosity of the sealant, are also a factors influencing the microleakage, and if a proper application method is followed, it can increase the length of resin tag and thus improve the efficiency of the sealant in preventing caries.

**Conclusion**

This study clearly indicated that, there does exists a relationship between the resin tag length and microleakage, and the longer the resin tags formed, the lesser is the microleakage, and the better will the cariostatic action of the pit and fissure sealant be. The viscosity too plays a role in the formation of these all important resin tags. The lower viscosity sealants are better. Also, with the newly developed hydrophilic sealant Embrace wetbond, it is now possibly to go ahead and seal the newly erupted teeth that were previously left unprotected due to moisture control problems.
Acknowledgments
The authors would like to acknowledge Dr. Sadashiva Shetty, Principal of Bapuji Dental College; for his support in making this study a success, the Indian institute of sciences for their gracious support in the utilization of the scanning electron microscope; and, last but definitely not the least, Dr.Thimmashetty, Head of Department, department of pharmaceutics, Bapuji Pharmacy College, for his help.

Footnotes
Source of Support: Nil.
Conflict of Interest: None declared.

References

Articles from Contemporary Clinical Dentistry are provided here courtesy of Medknow Publications

Formats:

• Article
  • PubReader
  • ePub (beta)
  • Printer Friendly

Related citations in PubMed

• Microleakage and SEM evaluation of fissure sealants placed by use of self-etching priming agents.[J Dent. 2004]
• Comparisons of in vitro penetration and adaptation of moisture tolerant resin sealant and conventional resin sealant in different fissure types.[Chin J Dent Res. 2013]
• Microleakage evaluation of pit and fissure sealants done with different procedures, materials, and laser after invasive technique.[J Clin Pediatr Dent. 1999]
• Effect of sealant viscosity on the penetration of resin into etched human enamel [Oper Dent. 2000]
• Pit and fissure sealant: review of the literature [Pediatr Dent. 2002]

See reviews...See all...

Cited by other articles in PMC
• Moisture-tolerant resin-based sealant: A boon [Contemporary Clinical Dentistry. 2013]

See all...

Links
• MedGen
• PubMed

Recent Activity
ClearTurn Off
• Comparative evaluation of the length of resin tags, viscosity and microleakage o...

See more...
• Influence of storage solution on enamel demineralization submitted to pH cycling [J Appl Oral Sci. 2004]
• Time of development of occlusal and proximal lesions: implications for fissure sealants [J Public Health Dent. 1986]
• Degree of microleakage of some pit and fissure sealants: an in vitro study [Int J Paediatr Dent. 1996]
• Degree of microleakage of some pit and fissure sealants: an in vitro study [Int J Paediatr Dent. 1996]
• In vitro investigation of the tensile bond strengths of a chemically initiated and a visible light-initiated sealant with SEM observations [Pediatr Dent. 1987]
• Properties of sealants containing bis-GMA and various diluents [J Dent Res. 1978]
• Pits and fissures: relative space contribution in fissures from sealants, prophylaxis pastes and organic remnants [Aust Dent J. 2003]
• Penetration and microleakage of dental sealants in artificial fissures [J Dent Child (Chic). 2004]
• **Properties of sealants containing bis-GMA and various diluents.** [J Dent Res. 1978]

• **Prevalence of dental caries and enamel defects in Connecticut Head Start children.** [Pediatr Dent. 2003]

• **The association between developmental enamel defects and caries in populations with and without fluoride in their drinking water.** [J Public Health Dent. 1996]

• **Influence of storage solution on enamel demineralization submitted to pH cycling.** [J Appl Oral Sci. 2004]

• **Penetration of acid solution and gel in occlusal fissures.** [J Am Dent Assoc. 1987]

• **Evaluation of pumice, fissure enameloplasty and air abrasion on sealant microleakage.** [Pediatr Dent. 2002]

• **Success with pit and fissure sealants.** [Dent Today. 2005]

• **A 5-year evaluation of two glass-ionomer cements used as fissure sealants.** [Community Dent Oral Epidemiol. 2003]

• **Review Sealants revisited: an update of the effectiveness of pit-and-fissure sealants.** [Caries Res. 1993]

• **Review Pit and fissure sealant: review of the literature.** [Pediatr Dent. 2002]

• **Evaluation of pumice, fissure enameloplasty and air abrasion on sealant microleakage.** [Pediatr Dent. 2002]

• **SEM analysis of sealant penetration in posterior approximal enamel carious lesions in vivo.** [J Adhes Dent. 2008]

• **Length of resin tags in pit-and-fissure sealants: all-in-one self-etching adhesive vs phosphoric acid etching.** [Compend Contin Educ Dent. 2008]

• **Pits and fissures: relative space contribution in fissures from sealants, prophylaxis pastes and organic remnants.** [Aust Dent J. 2003]

• **Effect of sealant viscosity on the penetration of resin into etched human enamel.** [Oper Dent. 2000]

• **Flow characteristics and sealing ability of fissure sealants.** [Oper Dent. 2000]

• **Flow characteristics and sealing ability of fissure sealants.** [Oper Dent. 2000]

• **Effect of sealant viscosity on the penetration of resin into etched human enamel.** [Oper Dent. 2000]

• **Properties of sealants containing bis-GMA and various diluents.** [J Dent Res. 1978]

• **Flow characteristics and sealing ability of fissure sealants.** [Oper Dent. 2000]

• **Penetration and microleakage of dental sealants in artificial fissures.** [J Dent (Chic). 2004]

• **Review Caries-risk assessment.** [Int Dent J. 1999]

• **In vitro assessment of marginal leakage of sealants placed in permanent molars with different etching times.** [ASDC J Dent Child. 1984]
- Microleakage of sealants after conventional, bur, and air-abrasion preparation of pits and fissures. [Pediatr Dent. 1998]

- Degree of microleakage of some pit and fissure sealants: an in vitro study. [Int J Paediatr Dent. 1996]

- Penetration and microleakage of dental sealants in artificial fissures. [J Dent Child (Chic). 2004]

- Marginal seal of sealant and compomer materials with and without enameoplasy. [Int J Paediatr Dent. 2002]

- In vitro assessment of marginal leakage of sealants placed in permanent molars with different etching times. [ASDC J Dent Child. 1984]

- Review The challenges of validating diagnostic methods and selecting appropriate gold standards. [J Dent Res. 2004]