RESEARCH ARTICLE

An *In Vitro* Study of Three Types of Pit and Fissure Sealants for Viscosity, Resin Tag, and Microleakage: A Scanning Electron Microscope Study

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Abstract

Introduction: One of the important techniques for prevention of dental caries is the application of pit and fissure sealants. The sealant success depends on not only adhering firmly to the enamel surface, but also isolation of pits and fissures from the rest of the oral environment.

The study was conducted to evaluate and compare three pit and fissure sealant materials for viscosity, microleakage, and resin tag length.

Materials and methods: Sixty premolars were selected for the study. The teeth were randomly divided into three groups. Group I: type VII GIC, group II: Delton FS, group III: Delton. Teeth were prepared by pumice prophylaxis and etched with 37% phosphoric acid. The respective pit and fissure sealants were applied. Teeth were stored in 1% methylene blue dye and sectioned mesiodistally into two halves. The microleakage was assessed using stereomicroscope and resin tag length using scanning electron microscope (SEM). Brookfield's viscometer was used to measure the viscosity.

Result: Viscosity was lowest for Delton and highest for type VII GIC. Microleakage was seen least with the Delton (0.0%) sealant, followed by Delton FS (20.0%), and highest in Type VII GIC (85.0%) in the scoring criteria of 3 and 4 and the mean length of the resin tags was least for Type VII GIC was 3.79 ± 2.58 µm, followed by Delton FS was 5.60 ± 2.12 µm, and highest for Delton was 7.27 ± 3.43 µm. The relation between viscosity, resin tag length, and microleakage was negative. As the viscosity gets lower, the more long the resin tags formed and the microleakage decreased. Delton pit and fissure sealant had lowest viscosity and microleakage scores while longest resin tag lengths.

Conclusion: Delton with the lowest viscosity and microleakage appears to be a more suitable pit and fissure sealant compatible with residual moisture and ideal for use in children, where isolation is a problem.

Keywords: Dental caries, Microleakage, Pit and fissure sealant, Resin tag length, Viscosity.

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Introduction

The caries incidence is very much dependent on the type of tooth surface like the occlusal surface has more caries risk as compared to smooth surfaces.[1] The tooth decay can be restricted by application of the sealants. The isolation of the pit and fissures from the rest of the oral tissues determines the success of a sealant. The sealant acts as a physical barrier in preventing oral bacteria and dietary carbohydrates from creating the acid conditions that result in caries.[2] The resin tag forms a bonding which is mechanically interlinked between the resin material and the tooth surface. The penetration of sealant is enhanced by viscosity, thus helping in retention.[3]

Rafatjou et al. stated that prevention of tooth caries is easier when there is application of pit and fissure sealants. The effectiveness of pit and fissure needs more precision and regular checkups.[4]

Prabhakar et al. study stated that the direct relationship between the resin tag length and microleakage, and the more longer the resin tags are, the lesser will be the microleakage, and more caries preventive action of the pit and fissure sealants.[2] The viscosity also plays an important role in the formation of the resin tags.[2] The good sealants have lower viscosity.

Retention of sealant is extremely important in fissure sealant therapy. The deeper penetration of the pit and fissure sealants into the enamel surface increases the retention of pit and fissure sealants. If the sealants are placed, maintained, and reapplied when necessary, the children could have been caries-free. A reapplication every 6 months, if needed, is recommended.[5]

The aim of the study is evaluation of the relationship between three different types of sealants and their properties of viscosity, resin tag length, and microleakage.

Materials and Methods

The *in vitro* study was conducted on 60 permanent premolars which were extracted either due to orthodontic reason or for periodontal reason. The extracted teeth were free from any caries infection.

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Sealant buttons were formed on either surface of occlusal surface of extracted tooth.

Test materials—Table 1 and Fig. 1.

Equipments used—Fig. 2.

- Stereomicroscope (Birbal Sahni Institute of Palaeosciences, Lucknow) (manufactured by Feica M 8).
- Scanning electron microscope (Birbal Sahni Institute of Palaeosciences, Lucknow) (manufactured by LEO 430).
- Brookfield's viscometer (School of Pharmacy, Babu Banarasi Das University, Lucknow) (manufactured by Brookfield).
- Thermocycling unit (Central Drug Research Institute, Lucknow) (manufactured by Eppendorf).

The selected teeth samples were cleaned with ultrasonic scalars to remove impurities and any staining and calculi on tooth surfaces and after that the teeth were washed thoroughly and stored in thymol solution at room temperature.

The teeth samples were divided in three equal groups: group I, group II, and group III. Each group having 20 samples of teeth:

- **Group I:** in group I, radio opaque glass ionomer cement type VII.
- **Group II:** in group II, light cure fluoride release Delton FS.
- **Group III:** in group III, self-cure nonfluoride release Delton.

Application of Sealant

Procedure for Type VII GIC and Delton Fissure Sealants

A total of 37% phosphoric acid for 30 seconds was applied on the occlusal surface of teeth to etch and it was rinsed with water. To get a frosty white appearance the teeth were air-dried. After air drying the type VII GIC and Delton sealants were applied and self-cured.

Procedure for Delton FS Sealant

Similar to earlier application procedure the teeth were etched with 37% of phosphoric acid and then air-dried for frosty appearance and then after the Delton FS sealant was applied and light cured for 20 seconds. The prepared teeth were stored in distilled water.

Viscosity Measurement

To check the viscosity of the sealant, it was firstly diluted into the methyl methacrylate monomer. Initially, the monomer viscosity was noted with help of Brookfield's viscometer in 10 mL test tube 1 mL of the sealant added along with methyl methacrylate and placed in Brookfield's viscometer and viscosity was measured in centipoises.

Microleakage Testing (Fig. 3)

The teeth were coated with double layer of nail varnish excluding the occlusal surface; for each group a different color nail varnish (purple, green, orange) was used to differentiate each of them easily.

The material was then applied as the manufacturer's indication on the occlusal surface. All the teeth samples of different groups were kept inverted in 1% methylene blue for 24 hours, then the sample were taken out of the solution and excess dye was removed by gentle brushing. All the three groups were then subjected to different thermocycling range of 5°C, 37°C, 55°C, and 37°C for 500 cycles with a dwell time of 15 seconds.

Each tooth was sectioned into two portions with the help of 0.02 mm thickness diamond wheel bur. The section was made longitudinally in a mesiodistal direction. After removing the root portion, the first half was examined under stereomicroscope to check the microleakage while the second half was tested for resin tag.

Table 1: Material profile

Sl. no	*Materials*	*Description*	*Batch no.*	*Manufacturer*
1.	Type VII GIC	Radio opaque glass ionomer protection and stabilization material Self-cure	Lot-1209041	FUJI GC Asia Dental Pte Ltd, India
2.	Delton FS	Light cure Fluoride release	Lot-110219	Dentsply International, York, PA
3.	Delton	Self-cure Nonfluoride release	Lot-131015	Dentsply International, York, PA

Figs 1A to C: Test materials used in the study. (A) Type VII GIC; (B) Delton; (C) Delton FS

Scoring Criteria for Microleakage Evaluation

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

Resin Tag Measurement

The second half of the section tooth was stored in normal saline after polishing and etching with 37% phosphoric acid. The sectioned tooth was dried under the heat lamp for 10 minutes (Fig. 4) after mounting on the stub with the help of nonconductor tape made of carbon. The dried tooth was placed inside an ion spluttering device (Fig. 4) for 30 minutes using vacuum evaporation at 200–300 A, the spluttered section was placed inside the SEM and photographs were obtained.

All the observations were recorded and subjected to statistical analysis.

Results

The obtained values were represented as mean ± standard deviation and range values, and were statistically analyzed using one-way analysis of variance for multiple group comparison, *post hoc* Tukey's test for group wise comparison, Pearson's correlation analysis, regression analysis for viscosity comparison, and the Mann–Whitney *U* test for multiple group comparison of the microleakage scores.

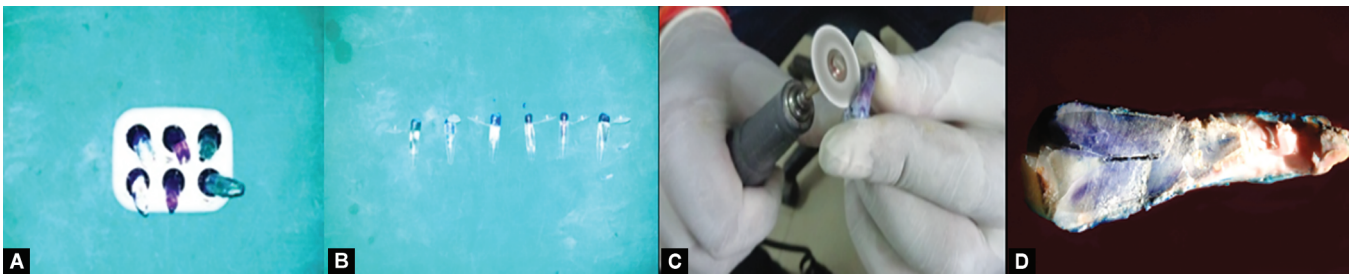
Assessing the viscosity of all three groups at different RPM (Table 2), Pearson's correlation analysis revealed significant and positive (direct) correlation between revolutions per minute (RPM) and viscosity of Delton, Delton FS, and Type VII GIC with the highest being in both Delton FS ($r = 0.91$, $p < 0.01$) and Delton ($r = 0.91$, $p < 0.01$) followed by Type VII GIC ($r = 0.86$, $p < 0.05$) indicating that as RPM increases, viscosity may also increase.

Further, regression analysis (Table 3) showed that rate of increase (regression slope: b) in viscosity with increase in RPM was highest in Type VII GIC (b = 4.90 centipoises/RPM) followed by Delton (b = 4.49 centipoises/RPM) and Delton the least (b = 3.59 centipoises/RPM) (Fig. 5).

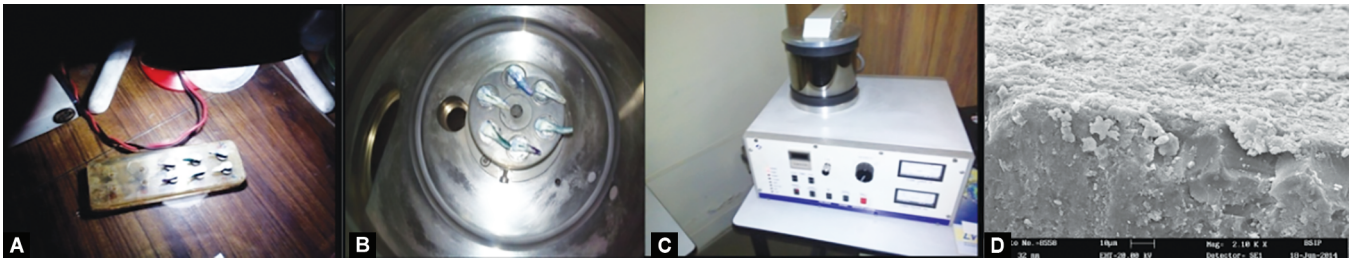
In Table 4, comparing the microleakage scores of three groups, χ^2 test revealed significantly different scores among the groups ($\chi^2 = 54.45$, $p < 0.001$). In other words, scores 3 and 4 both were significantly higher in both Delton FS (20.0%) especially Type VII GIC (85.0%) as compared to Delton (0.0%) (Fig. 6).

The resin tag lengths of three groups (Type VII GIC, Delton FS, and Delton) are summarized in Table 5. The resin tag lengths in Delton FS, Delton, and Type VII GIC ranged from 2.5 to 10.0 µm, 1.0 to 13.0 µm, and 0.2 to 7.8 µm, respectively with mean (± SD) 5.60 ± 2.12 µm, 7.27 ± 3.43 µm, and 3.79 ± 2.58 µm, respectively. The mean resin tag length of Delton was the highest followed by Delton FS and Type VII GIC, the least (Type VII GIC < Delton FS < Delton) (Figs 7 and 8).

Figs 2A to D: Equipments used in the study. (A) Stereomicroscope; (B) Scanning electron microscope; (C) Viscometer; (D) Thermocycling unit



Figs 3A to D: (A and B) Sample preparation; (C) Section cutting; (D) Stereomicroscope image of dye penetration for microleakage testing



Figs 4A to D: (A) Section tooth mounting; (B and C) Gold spluttering; (D) Scanning electron microscope image of resin tag

Table 2: Viscosity (centipoises) of three groups at different RPM

RMP	*Type VII GIC*	*Delton FS*	*Delton*
10	0	0	0
20	0	0	0
50	400	0	0
100	400	400	320

Tables 3: Correlation and simple linear regression between RPM and viscosity of three groups

Sealants	*Correlation (r)*	*Coefficient of determination (R^2)*	*Regression intercept (a)*	*Regression slope (b)*	*$y^{\#} = a + bx^{\$}$*
Type VII GIC	0.86	0.73	–20.41	4.90	y = –20.41 ± 4.90x
Delton FS	0.91	0.82	–102.04	4.49	y = –102.04 + 4.49x
Delton	0.91	0.82	–81.63	3.59	y = –81.63 ± 3.59x

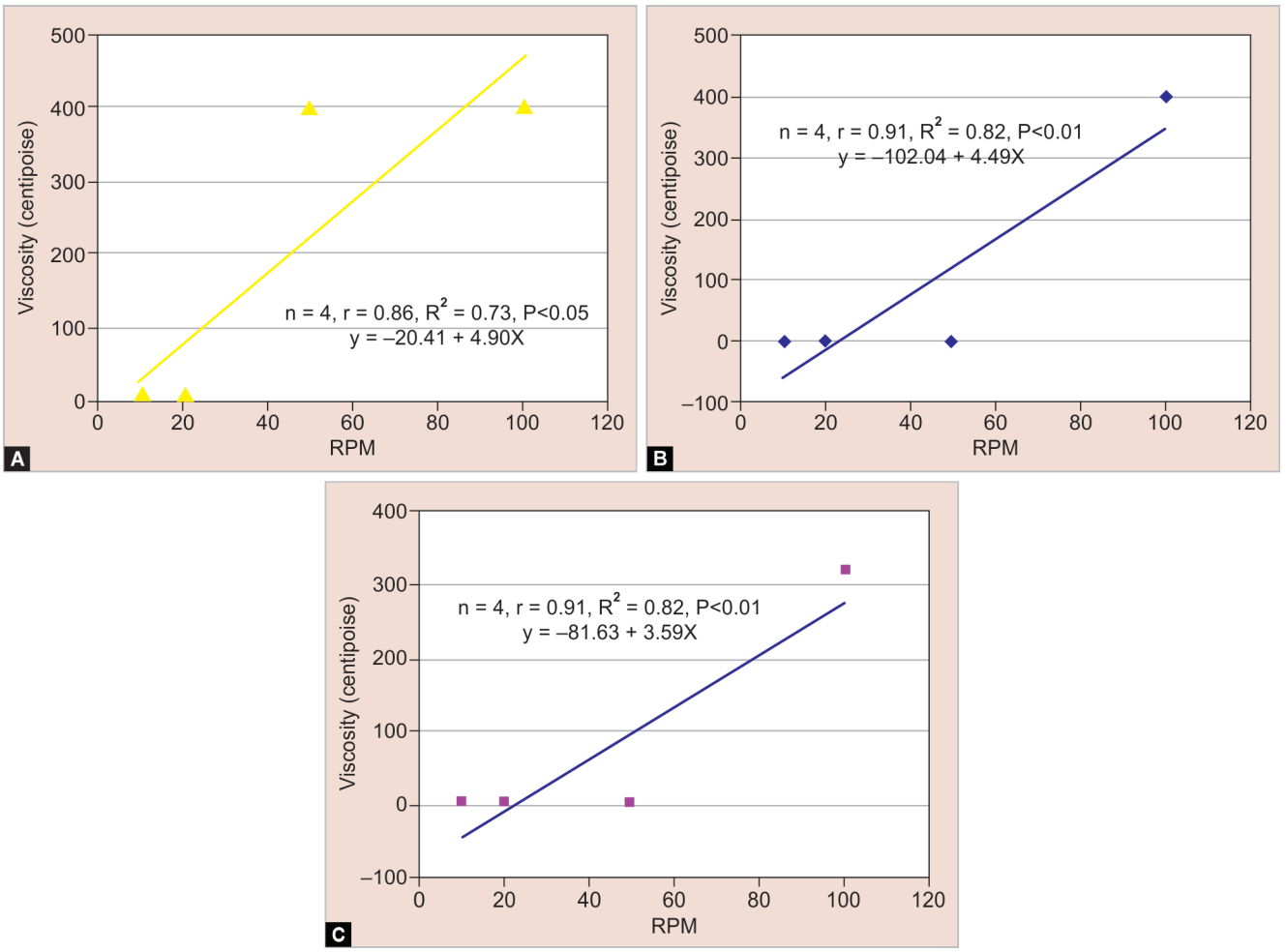


Fig. 5: Correlation and best fit regression between RPM and viscosity in type VII GIC, Delton FS, and Delton

Table 4: Microleakage score of three groups

Microleakage score	*Type VII GIC (n = 20) (%)*	*Delton FS (n = 20) (%)*	*Delton (n = 20) (%)*	*χ2 value (DF = 8)*	*p-value*
0	0 (0.0)	1 (5.0)	11 (55.0)	54.45	<0.001
1	0 (0.0)	10 (50.0)	8 (40.0)		
2	3 (15.0)	5 (25.0)	1 (5.0)		
3	7 (35.0)	3 (15.0)	0 (0.0)		
4	10 (50.0)	1 (5.0)	0 (0.0)		

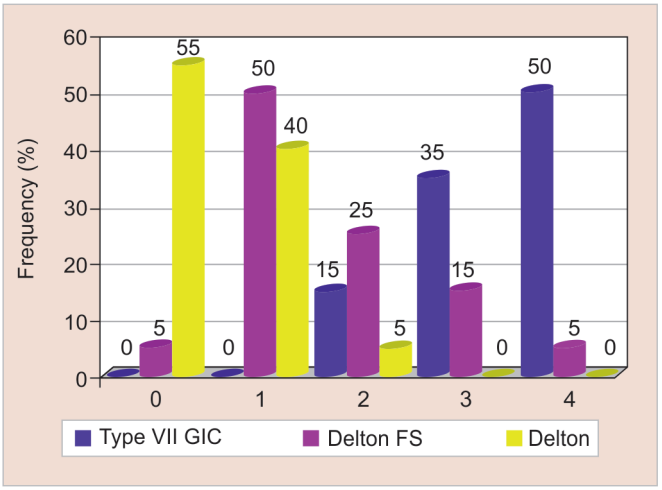


Fig. 6: Microleakage comparison of three groups

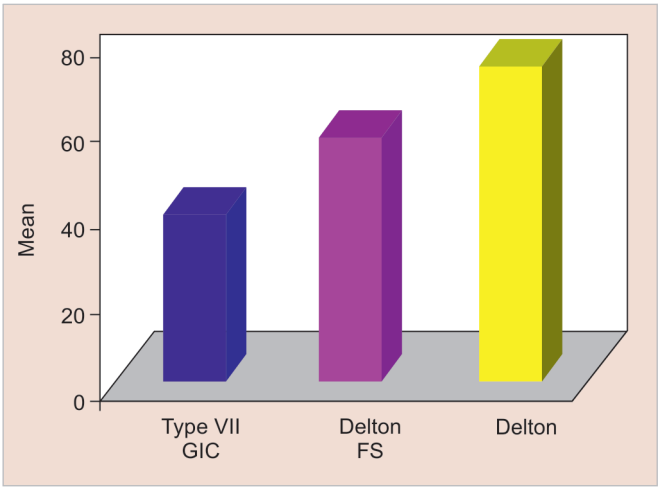


Fig. 7: Resin tag length comparison

Table 5: Resin tag length (mean ± SD) of three groups

Type VII GIC (n = 20)	*Delton FS (n = 20)*	*Delton (n = 20)*
3.79 ± 2.58 (0.2–7.8)	5.60 ± 2.12 (2.5–10.0)	7.27 ± 3.43 (1.0–.0)

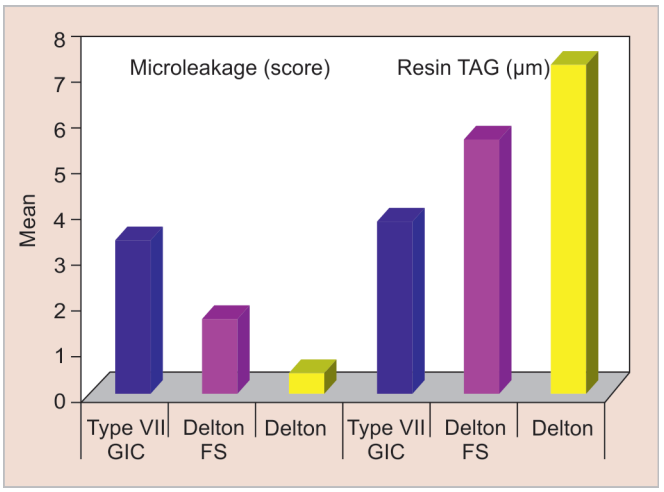


Fig. 8: Comparative study of three groups: viscosity, microleakage, and resin tag length

Discussion

Due to the unique morphology of occlusal surface of posterior teeth, these are more vulnerable to carious lesions and due to varying anatomy, the debridements are not easy to clean through normal tooth brushing and the bristles are too wide and large to penetrate into the fissures which leads to occurrence of frequent dental caries.[4,6] Therefore, to prevent initiation of dental caries in these fissures, the concept of pit and fissure sealant evolved.[7]

The application of pit and fissure sealants forms a mechanical barrier between tooth surfaces and bacteria and thus prevents further development of caries.

There are many evidences which show that pit and fissure sealants are capable of caries prevention like Beauchamp et al.,[8] Oong et al.,[9] and Bakhshandeh et al.[10]

A pit and fissure sealant is effective in preventing dental caries, only when it is retained in the fissures. Hence, retention becomes an important factor, influencing the efficacy of the pit and fissure sealant. The micromechanical interlocking between the resin and the enamel is the reason of retention in the resin-based pit and fissure sealant.[4] Monomer in the resin-based material polymerizes and the material becomes interlocked with the enamel surface.[4] Whereas retention in Type VII GIC is achieved through ionic bonding.

According to Subramaniam et al.,[11] the routine clinical use of resin-based sealant Delton and glass ionomer sealant Type VII GIC at the end of 1 year showed complete retention of 14% of resin-based sealant as compared to only 0.9% of glass ionomer cement. This difference was highly significant. One of the important factors for a pit and fissure sealant to be effective is its retention in the fissures. The interlocking between the resin and the enamel at microlevel is the reason of retention in the resin-based pit and fissure sealant.[4] The polymerization of the monomer in the resin-based materials cause interlocking with the enamel surface whereas retention in Type VII GIC is achieved through ionic bonding.

According to Subramaniam et al.,[11] the regular use Type VII GIC for a continuous 1 year showed retention up to 14% and the resin-based sealants showed only at the end of 0.9% of glass ionomer cement with a statistically highly significant value.

In our study, the mean length of the resin tags of Type VII GIC was 3.79 ± 2.58 µm, for Delton FS was 5.60 ± 2.12 µm, and for Delton was 7.27 ± 3.43 µm.

Gomez et al.[12] compared the resin tag length in four different groups which were differentiated according to bonding system. They concluded that the bonding system having longer resin tag experiences lesser microleakage.

Viscosity is one of the important factors which influences penetration of sealants other than surface tension. It can be defined as the resistance of a liquid to flow. The more the viscosity of a liquid will be the lesser will be its flow. The unit of viscosity is mega Pascal per second or centipoises. The higher viscosity leads to poor penetration of the resin which reduces the retentive property of the resin while the resins having lower viscosity flows easily and penetrates deeply into the tooth surface and forms greater bond.[4]

Percinoto et al.[7,13] mentioned that low viscosity sealants penetrate with greater potential into the fissures and microporosities developed by phosphoric acid were corroboratory in our study. On the contrary to our study, Barnes et al. concluded from their research that the sealing ability is not affected by the viscosity and flow property.[14]

The study conducted showed that the sealant with high fluoride release is Type VII GIC followed by Delton FS which also releases fluoride showed highest viscosity. The least viscous was the Delton (nonfluoride releasing). There was statistically significant difference of viscosity seen in Type VII GIC ($r = 0.86$, $p < 0.05$), Delton FS ($r = 0.91$, $p < 0.01$) vs Delton ($r = 0.91$, $p < 0.01$). No statistically significant difference of viscosity was seen between Delton and Delton FS.

Microleakage can be defined as the pathway formed between the restoration margin and the tooth surface for the penetration of bacteria and toxins. Microleakage causes bacterial penetration which leads to the carious process thus it lessens the resin property to form a proper seal to prevent caries. If restoration is sealed properly, then the bacterial invasion is not possible.[15,16]

In the present study, perfect seal was judged on the basis of penetration of the dye as the more dye will penetrate the weaker the seal will be. The presence of dye under etched enamel surface shows the property of microleakage. All the sealants used in the study showed some degree of microleakage which was acceptable to previous studies.[7,17,18]

Park et al.[19] reported no significant differences in microleakage between unfilled (Delton), filled (primashield), and fluoride-releasing sealants (fluroshield), and showed the mean shear bond strength of Delton (12.18 ± 2.70).

In the present study, the least microleakage was seen with the Delton (0.0%) sealant, followed by Delton FS (20.0%) and highest in Type VII GIC (85.0%) in the scoring criteria of 3 and 4. Due to phenomenon of closed end capillary or isolated capillaries there is incomplete penetration of sealant into the fissure systems. The gold standard for microleakage studies is stereomicroscope, hence, it was implemented in this present study. As SEM produces high resolution images it can easily measure the resin tag length with minor details as low as 1–5 nm.

In our study, viscosity, microleakage, and resin tag measurements were carried out on the same tooth to minimize substrate variations, as these results are valid only in *in vitro* conditions.

Every pit and fissure sealants have different properties to act accordingly in different environments, different types of fissures, preparation of fissures, etching and conditioning of enamel surface, bonding agent application, and contamination of prepared surfaces of fissures. The proper procedure of sealant application leads to prevent microleakage and increases the resin tag length and prevents the tooth surface from bacterial invasion.[4]

Conclusion

It was concluded from the study that there is a direct relationship between the resin tag length and microleakage, and the more longer the resin tag length the lesser microleakage will be and lesser will be the bacterial invasion on the tooth surface which will prevent the further caries. The viscosity plays an important role in the formation of these resin tags. The lower the viscosity of the sealants the more deeper it penetrates and forms a greater bond to the tooth surface. But the endeavor for better material as sealant is desired.

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