ORIGINAL RESEARCH PAPER

Dentistry

COMPARATIVE EVALUATION OF DIFFERENT PIT AND FISSURE SEALANTS ON STREPTOCOCCUS MUTANS COUNT IN SALIVA OF CHILDREN: AN IN-VIVO STUDY

KEY WORDS:

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ABSTRACT

The aim of this study was to evaluate and compare the effects of different pit and fissure sealants, namely ClinPro Sealant (3M ESPE) and Helioseal-F (Ivoclar Vivadent) on the Streptococcus mutans count in the saliva of children. Fourty (40) caries free children in the age group of 6-12 years with erupted permanent molars were selected and following complete oral prophylaxis the materials were applied according to the manufacturer's instructions. Saliva samples were collected prior to application, immediately after application, one month after application, three months after application and six months after application. These samples were cultured on Mitis salivarius plates and colony counts of Streptococcus mutans were recorded. Reduction in Streptococcus mutans count over the period of study was observed in all the cases and the effectiveness of these pit and fissure sealants were comparable. Both the pit and fissure sealants - ClinPro Sealant (3M ESPE) and Helioseal-F (Ivoclar Vivadent) - are equally effective in reducing the salivary Streptococcus mutans in children and preventing the caries.

INTRODUCTION

Dental caries is a complex microbial disease due to the interaction between host, microflora and sugar over a period of time. The demineralisation of the tooth surface takes place due to the acid produced by the bacteria present on the tooth surface.[1]However, the influence of Streptococcus mutans on initiation of dental caries cannot be overlooked. The reduction in Streptococcus mutans count is very essential in the prevention of caries formation. Streptococcus mutans play a significant role in the initiation and progression of dental caries. [1]Streptococcus mutans adapts to environmental low pH and thus increase their rate of acid production and drive the pH still lower resulting in a cariogenic plaque.[2] Streptococcus mutans have been measured in saliva of children from different background and they have been found to correlate with patient's caries activity levels.[3]

Occlusal surfaces of tooth are highly susceptible to dental caries due to the varying anatomical irregularities like pits and fissures. For this reason, use of pits and fissures have been advocated to prevent the formation of caries in these areas. Pit and Fissure sealants are substances that can penetrate into the micro porosities and form a continuous and resistant film which adapts and adheres to the irregularities perfectly and hinder the dental plaque formation. The sealing capacity of a pit and fissure sealant is further improved with the use of etchant and a bonding agent to further reinforce the bond between the tooth and the material. [4],[5],[6]

Various studies have been done to evaluate the physical properties of pit and fissure sealants but very limited studies have been done on the decrease in microbiological load after the application of fluoridated pit and fissure sealants. Apart from that, no study has been done, to the best of my knowledge, to evaluate the effect of pit and fissure sealant on Streptococcus mutans count in saliva to determine which material is superior in reducing the Streptococcus mutans count in saliva of children over a period of 6-months.

A systematic study was planned to evaluate and compare the effects of different pit and fissure sealants- ClinPro Sealant (3M ESPE) and Helioseal-F (Ivoclar Vivadent) on the *Streptococcus mutans* count in the saliva of children. The objective of this study was to evaluate and compare the *Streptococcus mutans* count in saliva of children before application (control) to immediately after application, 1-month,3-months and 6-months after application of pit and fissure sealants so as to determine the effect on the change in *Streptococcus mutans* post-application.

METHODS AND MATERIAL

The study was approved by the institutional ethics committee. The study was conducted on 40 children reporting to Dental Camps organised by our Department. A thorough check-up of the patients was done using mouth mirror and probe and forty caries free patients were selected after obtaining informed consent from the parents/ guardians of the children. To prevent operator bias, the children were randomly divided into two groups using numeric coding. The inclusion criteria were: caries free children in the age group of 6 to 13 years with overall good oral health and hygiene and fully erupted first permanent molars. Children with the history of pit and fissure sealants application or fluoride treatment in the past 6 months, carious teeth and children whose parents/guardians refuse to give informed consent were excluded from the study.

The baseline saliva sample was collected prior to any treatment by asking the child to drool out 1ml of whole unstimulated saliva in sterile collection tubes by tilting their head down and pooling the saliva in their mouth after which the saliva was slowly dripped into the sample collection tubes passively.

The material was applied on the permanent molars after complete scaling and polishing using prophylactic paste. Once the oral prophylaxis was done, the tooth was isolated with the use of cotton rounds and high-volume saliva ejector. The occlusal surface of the permanent tooth was etched using 37% phosphoric acid followed by washing it was water and drying after which a white frosty surface was observed. This was followed by the application of pit and fissure sealants on the occlusal surface of the permanent molars under proper isolation and was cured with curing light for 60 seconds. Following this, the saliva sample was collected using the same drooling method in sterile collection tube. The saliva samples were transported to the laboratory in sterile ice box to help maintain the viability of the test organism, immediately after collection and processed on the same day. The samples were stored at the temperature of -20°C to prevent the loss of viability of the test organism.

The saliva sample was buffered in 0.05 M phosphate solution to a dilution of 1/20 and agitated on a vortex for 30 seconds. 100 µL of this solution was used for inoculation on by Mitis Salivarius Agar plates. The plates were incubated at 37°C for 48 hrs. After 48 hrs, colony characteristics were studied and the number of colony forming units of Mitis Salivarius (CFU/ml) were counted with the use of manual colony counter. The count of each sample was tabulated.

The sample collected after the completion of the treatment was also analysed for the *Streptococcus mutans* count in a similar way as the pre-treatment sample. Likewise, all the

saliva samples -1-month, 3-months and 6-months post treatment samples, were collected in sterile collection tubes and transported and inoculated on the same day of collection and the result was obtained after 48 hours.

RESULTS

Table 1 presents the mean and standard deviation of the Streptococcus mutans counts recorded at five different occasions (before application, immediately after the application, one-month after the application, three-months after the application and six-months after the application of the two sealants) from the samples of 20 children in each group. It was observed from this table that all the groups showed a decrease in the Streptococcus mutans count over the period of study. Further, it is also observed that there is marked reduction in Streptococcus mutans count in saliva of children over a period of 6-months but the reduction was more prominent in the third month after application.

Table 1: Mean and standard deviation (SD) of the Streptococcus mutans counts of the study groups at different time intervals

Time of data collection	Group 1: Clinpro Sealant (Mean±SD)	Group 2: Helioseal F Sealant (Mean±SD)
Before Application	151.30± 83.36	175.10± 47.64
Immediately After	93.95± 77.52	65.20± 43.59
1st month	66.90± 60.11	56.95± 40.91
3rd month	47.20± 37.68	29.85± 21.97
6th month	23.50± 15.95	13.81± 12.80

Table 2: Comparison of the two pit and fissure sealant groups (Group 2.1 Clinpro Sealant and Group 2.2 Helioseal F Sealant) at different time intervals.

Time Interval	Pearsons' correlation coefficient (R)	Significance
Before Application	0.157	0.507
Immediately After	0.281	0.230
1 Month After	0.032	0.895
3-Months After	0.441	0.052
6-Months After	0.369	0.109

Table 2 shows the correlation analysis between the two sealants. The correlation between the Streptococcus mutans counts of the samples taken before the application of sealant, immediately after the application, one-month post application, three months post application and six-months post the application of pit and fissure sealant. It was observed that there is reasonable correlation between the Streptococcus mutans count of the samples collected after three and six months of applications of sealants. However, there was no statistically significant difference in the effectiveness of the two pit and fissure sealants in the given time period.

DISCUSSION

Streptococcus mutans is a bacterium which is considered as a primary causative agent of dental caries as it is one of the primary colonisers on the surface of the tooth which not only attacks the tooth surface by the production of calcium depleting acids but also helps other bacterium to adhere to the surface of the tooth and cause further demineralization and improve the resistance of the plaque.

Pit and Fissure sealants are an acceptable method for the reduction in the caries incidence as it inhibits the colonisation of the caries forming bacteria on the most susceptible surface of the tooth, the pit and fissures. In the present study, the pit and fissure sealants- Clinpro sealant and Helioseal F sealants were used as the study material for the reduction in the *Streptococcus mutans* count in the saliva of children which in turn would help in reducing the caries incidence on the occlusal surface in children. Both the sealants used in this study are resin-based, fluoride releasing sealants that micromechanically bind to the tooth structure and prevent the pit and fissure from getting invaded by the caries forming bacteria.

Shlomo Matalon et al, 2003 [8] evaluated the antibacterial property of four different pit and fissure sealants-Helioseal F, Ultraseal XT, Conseal F, Dyract Seal- using Direct Contact Test (DCT) and Agar Diffusion Test (ADT) and concluded that pit and fissure sealants are a state-of-the-art method for prevention of initial occlusal caries. Yildiz et al, 2004 [9] studied the retention and caries prevention effect of fluoride containing pit and fissure sealants in young population and concluded that the use of pit and fissure sealants is effective in reducing caries incidences. Although, the effect is related more to the quality of the sealant retention instead of the composition. Menon et al, 2007 [10] studied the antibacterial properties of two fluoride releasing and a non-fluoride releasing pit and fissure sealant with regard to Lactobacillus and Streptococcus mutans and concluded that only fluoride-based varnish amongst the selected materials for testing was capable in reducing the *Streptococcus mutans* count in the saliva. Hatem et al, 2008 [11] conducted a study to evaluate the retention rate, caries-preventive effect and long-term inhibitory effect of three different types of pit and fissure sealants on *Streptococcus mutans* counts and concluded that there was marked reduction in *Streptococcus mutans* count in saliva after using Resin-modified Glass ionomer cement, followed by Conventional Sealant and Flowable Composite. Hussain et al, 2008 [12] studied the role of fissure sealants in reducing the number of cariogenic bacteria in 6-8-year-old children and was concluded that pit and fissure sealants have a positive role in reducing the number of cariogenic bacteria in the children. Erdemir et al, 2013 [13] studied the retention rate and caries-prevention effect of a flowable composite compared to a conventional resin-based sealant in young population over 24-months period and concluded that flowable composite resin sealants showed better retention than conventional sealant when used in conjunction with etching and adhesive whereas both the sealants were effective in reducing the *Streptococcus mutans* count in the saliva. Muller-Bolla et al, 2018 [14] studied the effectiveness of resin-based sealant with and without fluoride placed in high-caries risk population with 3-6 caries in 2-year follow up. The study concluded that sealed tooth was less likely to develop caries than tooth which are not sealed and loss of sealant was not associated with increased risk of caries. All the above stated studies showed that there was a reduction in the microbial count after the application of the pit and fissure sealant however it must be noted that multiple studies also showed that the effect of the sealants was dependent not only on the chemical composition but also on the mechanical adherence of the material to the tooth surface. The adherence was however dependent on the method of application, use of proper isolation. In our study, there was no loss of the sealant material observed in any group in the follow up visits hence a much clearer picture of the effectiveness of the material in the test time was obtained.

CONCLUSIONS

The results of the present study indicated the reduction in Streptococcus mutans count in saliva due to the application of both of the pit and fissure sealants. Thus, the preventive materials have effective antimicrobial property which helps in reducing the incidence of dental caries. Further, the effects of these pit and fissure sealants are comparable to each other and hence it is difficult to predict the superiority of a given sealant. This may be attributed to the bond between the tooth structure and the material preventing the colonisation of the test organism on the susceptible surfaces of the tooth. Based on the comparisons of the two materials, satisfactory effect was shown by both the pit hence it can be concluded that, both the materials can be efficiently used on the tooth surfaces as preventive material to help decrease the incidence of dental caries in children

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