RESEARCH ARTICLE

Comparative Evaluation of Different Varnishes and Pit and Fissure Sealants on *Streptococcus mutans* Count in Saliva of Children

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Abstract

Aim: This study aims to evaluate and compare the effect of different varnishes: 3M ESPE Clinpro and Voco Profluorid and fissure sealants- 3M ESPE ClinPro and Ivoclar Vivadent Helioseal-F on the *S. mutans* count in the saliva of children.

Materials and methods: Eighty children of 6–12 years of age with no incidence of caries were selected and saliva samples were collected by drooling method after oral prophylaxis. The children were divided randomly into four groups and materials were applied accordingly. Saliva samples were obtained immediately after the procedure as well as 1-month, 3-months and 6-months posttreatment. Saliva samples were inoculated on Mitis salivarius agar and colony counts of *S. mutans* were obtained.

Results: This study showed that both the varnishes and pit and fissure sealants were equally effective in reducing the salivary *S. mutans* count in children in a time period of 6-months however pit and fissure sealants were more effective in preventing caries.

Conclusion: Both varnishes and pit and fissure sealants are equally effective in reducing *S. mutans* count in saliva.

Keywords: Sealant, *S. mutans*, Pit and fissures, Varnish.

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Introduction

Plaque is a tenaciously adherent material accumulating on the occlusal as well as smooth surface of teeth. A variety of endogenous microorganisms found in dental plaque are considered crucial to the initiation and progression of dental caries. They are mutans Streptococci, *Lactobacillus* species, *Actinomyces* species, non-mutans streptococci, and yeast. The virulent traits of the microorganisms are strongly associated with caries including the ability to produce acid and to sustain acid production at low pH levels. The sustained maintenance of pH levels and production of acids result in demineralization of calcified structure, formation and use of extra- and intracellular storage polysaccharides that allow the microorganisms to continuously produce acid even after the depletion of substrate, and formation of water insoluble glucans which aids in the accumulation of *S. mutans* in plaque as well as allow the substrate to diffuse to a deeper layer of the dental plaque biofilm.[1]

Longitudinal studies have shown a relative rise of *S. mutans* in plaque samples from tooth surfaces that become carious at a later stage. *S. mutans* have been measured in saliva of children from different background and they have been found to correlate with patient's caries activity levels.[2,3]

Fluorides have several caries-protective mechanisms of action. Topically, low levels of fluoride in plaque and saliva inhibit the demineralization of sound enamel and enhance the remineralization of demineralized enamel. Tencate reported fluoride concentration as low as 0.02–0.06 ppm has been shown to enhance remineralization when enamel specimens were subjected to *in-vitro* demineralization.[4] Fluoride also inhibits dental caries by affecting the metabolic activity of cariogenic bacteria.[5] Professionally-applied topical fluoride treatments are efficacious in reducing prevalence of dental caries.[6]

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Occlusal surfaces of tooth are susceptible to dental caries due to the varying anatomical irregularities which has minimal self-cleansing capacity and higher risk of caries development. Pit and fissure sealants have mechanical action actin on the tooth as it physically seals off the most susceptable areas of the tooth which are the pit and the fissures, leading to reduction in susceptibility toward caries and intern reduction in the microbial load.[7,8]

Various studies have been done to evaluate the effect of varnishes on *S. mutans* 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 *S. mutans* count in saliva as well as a comparative study between fluoride varnish and pit and fissure sealant to determine which method of preventive dentistry is superior in reducing the *S. mutans* count in saliva of children over a period of 6-months.

Hence a systemic study was planned to evaluate and compare the effects of different varnishes—ClinPro Varnish (3M ESPE) and Profluorid Varnish (Voco) and pit and fissure sealants—ClinPro Sealant (3M ESPE) and Helioseal-F (Ivoclar Vivadent) on the *S. mutans* count in the saliva of children.

Materials and Methods

The study was approved by the institutional ethical committee. Eighty children in the age group of 6–12 years reported to dental camps organized by the Department of Pediatric and Preventive Dentistry, Santosh Dental College and Hospital, Ghaziabad. Checkup of the patients was done using mouth mirror children and the cases were selected after obtaining informed consent from the parents/guardians. The selected patients were given codes to prevent operators bias and subjects were randomly divided into four groups—Group 2.1 ClinPro Varnish (3M ESPE), Group 1.2 Profluorid Varnish (Voco), Group 2 A ClinPro Sealant (3M ESPE), and Group 2.2 Helioseal-F (Ivoclar Vivadent).

The inclusion criteria: caries free children with overall good oral health and hygiene, and at least one permanent molars should be erupted in maxillary and/or mandibular arch. Children with existing carious teeth, and the history of pit and fissure sealants application or fluoride treatment in the past 6 months were excluded from the study.

The baseline saliva sample was collected prior to any treatment by asking the child to drool out 1 mL 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. Oral prophylaxis was done, the tooth was isolated with the use of cotton rounds and high-volume saliva ejector.

The varnish was applied using a single tufted brush on all the surfaces of the tooth and was cured using curing light for 30–60 seconds after which the patient was asked to pool the saliva in the mouth and collect it in the sterile tube using drooling technique.

Similarly, pit and fissure sealants were applied on the occlusal surface of the permanent molars under proper isolation and was cured with curing light for 60 seconds after which the saliva sample was collected using the same drooling method in sterile collection tube.

The samples were transported to the laboratory in sterile icebox 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 samples were coded and processed according to the adapted coding criteria to avoid operator bias. 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 hours. After 48 hours, colony characteristics were studied and the number of colony forming units of Mitis Salivarius (CFU/mL) of saliva were counted.

The sample collected after the completion of the treatment was also analyzed for the *S. mutans* count in a similar way as the pretreatment 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.

Statistical Analysis

The statistical analysis for this study was done using Statistical Package for Social Sciences Software, Version 23.0. Table 1 presents the mean and standard deviation of the *S. mutans* counts recorded at five different occasions (before application of the varnish/sealant, immediately after the application, 1-month after the application, 3-months after the application, and 6-months after the application) from the samples of 20 children in each group.

Table 2 presents the parametric statistics of *S. mutans* counts of samples taken for study the impact of varnishes. As the purpose was to compare two samples, Student's *t*-test was applied to test the probability that the samples come from a population with the same mean value. But the significance value indicates that the there is strong evidence that the impact of two varnishes are different. The effect of the varnishes is patient dependent, mostly on oral hygiene maintenance of the individual.

Table 3 presents the parametric statistics of *S. mutans* counts of samples taken for study the impact of sealants. As indicated earlier, Student's *t*-test was conducted to test the probability that the samples come from a population with the same mean value. But the significance value indicates that there is good evidence that the impact of two sealants are more pronoun after 3 and 6 months of their applications. However, in other cases, the impact of the two sealants is not statistically different.

Table 4 shows the comparison between the varnishes and sealants. It is obvious from the data that the effect of varnishes and sealants are not having any correlation as far as the number of *S. mutans* counts are concerned. This analysis indicates that the impact of varnishes and impact of sealants are quite different, independent of each other, and are more patient specific.

Table 1: The mean and standard deviation of the *S. mutans* counts of different study groups at different time intervals (BA: before application, IA: immediately after application, 1M: 1 month after application, 3M: 3 months after application, and 6M: 6 months after application)

	Mean mutant count for			
	Group 1: varnish		*Group 2: sealant*	
Time interval	*Group 1.1 Clinpro Varnish (3M ESPE)*	*Group 1.2 Profluorid Varnish (Voco)*	*Group 2.1 Clinpro Sealant (3M ESPE)*	*Group 2.2 Helioseal F Sealant (Ivoclar Vivadent)*
BA	392.80 ± 154.15	100.45 ± 40.20	151.30 ± 83.36	175.10 ± 47.64
IA	205.80 ± 126.34	61.25 ± 36.10	93.95 ± 77.52	65.20 ± 43.59
1M	121.10 ± 58.46	28.25 ± 25.52	66.90 ± 60.11	56.95 ± 40.91
3M	90.40 ± 48.29	14.30 ± 13.10	47.20 ± 37.68	29.85 ± 21.97
6M	69.80 ± 46.91	9.90 ± 8.35	23.50 ± 15.95	13.81 ± 12.80

Table 2: Parametric statistics (*t*-test) of *S. mutans* counts of the samples of varnishes (G1.1: Group 1.1 3M ESPE Clinpro Varnish, G1.2: Group 1.2 Voco Profluorid Varnish, BA: before application, IA: immediately after application, 1M: 1 month after application, 3M: 3 months after application, and 6M: 6 months after application)

Pair of varnish samples	*Mean*	*Standard deviation*	*t-test*	*Significance (p value)*
G1.1 BA–G1.2 BA	292.35	153.06	8.54	0.0001
G1.1 IA–G1.2 IA	144.55	136.38	4.74	0.0001
G1.1 1M–G1.2 1M	92.85	66.95	6.20	0.0001
G1.1 3M–G1.2 3M	76.10	53.02	6.42	0.0001
G1.1 6M–G1.2 6M	61.45	50.18	5.48	0.0001

Table 3: Parametric statistics (*t*-test) of *S. mutans* counts of the samples of sealants (G2.1: Group 2.1 3M ESPE Clinpro Sealant, G2.2: Group 2.2 Ivoclar Vivadent Helioseal F Sealant, BA: before application, IA: immediately after application, 1M: 1 month after application, 3M: 3 months after application, and 6M: 6 months after application)

Pair of sealant samples	*Mean*	*Standard deviation*	*t-test*	*Significance (p value)*
G2.1 BA–G2.2 BA	23.80	89.26	–1.192	0.2480
G2.1 IA–G2.2 IA	28.75	77.52	1.659	0.1140
G2.1 1M–G2.2 1M	9.95	71.64	0.621	0.5420
G2.1 3M–G2.2 3M	17.35	34.25	2.266	0.0350
G2.1 6M–G2.2 6M	10.70	16.81	2.847	0.0100

Table 4: Comparison between varnishes and pit and fissure sealants with relation to various time intervals using Pearson's test (BA: before application, IA: immediately after application, 1M: 1 month after application, 3M: 3 months after application, and 6M: 6 months after application)

	Mean		*Pearson's correlation coefficient (R)*	*Significance (p = 0.5)*
Time interval	*Group 1, varnish*	*Group 2, sealants*		
BA	241.62	163.20	0.040	0.806
IA	133.52	79.57	0.063	0.698
1M	74.67	61.77	0.052	0.749
3M	52.35	38.52	0.004	0.981
6M	39.85	18.65	0.090	0.580

Discussion

In this study, fluoride varnishes—Clinpro Varnish and Voco Profluorid varnish were used to assess the reduction in *S. mutans* count in the saliva of caries free children. The study sample group children were in the age group of 6–13 years old with at least one erupted permanent molar with the mean age of 10.03 ± 3.01 years. The selected subjects were randomly divided using Microsoft Excel software into four groups of 20 subjects each to prevent bias.

The unstimulated saliva sample was collected using a drooling method as a stimulated saliva sample would have the incorporation of other bacterial groups apart from *S. mutans* which was not desirable for our study and it would also dilute the sample which would not provide an accurate picture of the *S. mutans* count in the saliva.

S. mutans is a bacterium which is considered as a primary causative agent of dental caries as it is one of the primary colonizers 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.

Zicker investigated the effect of tropical fluoride varnish application on *S. mutans* count in plaque and saliva of school children and showed that a single application of fluoride varnish had no effect on incidence of *S. mutans* present in saliva and plaque of school children. This study was in opposition to the current study which showed that there was significant decline in the *S. mutans* count in saliva of children in 6-month study period.[9] There was a single application of the varnish done in the initial phases of the study with a pronoun effect seen in the test period of 6-months. Deepti et al. evaluated the effect of fluor protector varnish on *S. mutans* count in saliva of caries free children and found that it had profound effect on reduction in *S. mutans* count in a period of 24-hours.[10] Similarly Pitchika et al. studied the effectiveness of Clinpro varnish with regard to reduction in caries incidence and finally concluded that there was significant decrease in the caries prevalence after 2 years.[11]

Sourabh Badjatia et al. evaluated the effect of fluoride varnish on *S. mutans* count in saliva of 12-year old children and concluded that fluoride varnish can be used in cases with caries as an effective antibacterial agent against *S. mutans* to help in caries reduction.[12] Patel et al. compared fluoride varnish, chlorhexidine varnish, and fluoride varnish with CPP-ACP on salivary *S. mutans* level in saliva of children with mixed dentition and concluded that even though all the three varnishes showed a drop in *S. mutans* count in 6-months post application, chlorhexidine varnish showed to be more effective in reducing the *S. mutans* count in saliva of children in 6-months.[13] The results of all the above studies are in accordance with the present study however the target population

for our study was caries free children while this study selected children with caries and established the effectiveness of varnish on children with caries.

The present study also compared the effectiveness of varnishes to pit and fissure sealants in the reduction of the *S. mutans* count and it was observed that even though both the materials were effective in the reduction of *S. mutans* count but the reduction was more significant with the use of pit and fissure sealant than varnishes. This may be due to prolong contact of the material to the tooth surface whereas the application of varnish is subjected to daily brushing and depletion of the surface available fluoride moity. The effect of fluoride lasts for a specific period of time as it is a surface acting agent which shows its effect when in contact the susceptible tooth surface.

Conclusion

The conclusions derived from the results of this study are that both the varnishes and pit and fissure sealants were effective in reducing the *S. mutans* count when compared to the baseline and that pit and fissure sealants were more effective than fluoride varnish in reducing the *S. mutans* count in saliva of children. As this was the first study, further studies can reveal the long term effect of fluoride varnish and pit and fissure sealants on the *S. mutans* count in plaque of carious and caries free dentition. Based on the comparisons of the two materials, satisfactory effect is shown by both the materials hence it can be concluded that both the materials can be effectively used on the tooth surfaces as preventive material to help decrease the incidence of dental caries in children.

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