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Effect of Fluoride and Chlorhexidine Varnish on Biofilm formation of *Streptococcus mutans*

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Abstract

The study aimed at finding the action of Fluoride and Chlorhexidine varnish on the amount of biofilm produced by Streptococcus mutans in vitro. Streptococcus mutans isolated from the plaque of 30 patients prior to varnish treatment and following treatment were used for the study. Biofilm production was done by O' Toole and Kolter method and OD values were recorded spectrophotometrically at 48h. The same was repeated following 48h, 1 month and 3 months after varnish treatment. The amount of biofilm produced was evaluated. The results were compiled systematically and analysed using SPSS 17.0. Group comparison was done by ANOVA test and intergroup comparison at different time intervals was done by Bonferroni t test. Comparison between the groups was done by Tukey's test. Streptococcus mutans was isolated from all 30 of them after 1month and 3 months following treatment. There was a significant decrease in biofilm production by the isolates after treatment with varnish. This study suggests that there is gradual loss of effect of Fluoride and Chlorhexidine varnish on viability of Streptococcus mutans in dental plaque. Nonetheless it can decrease the biofilm producing property of the organism in vitro.

Keywords: Streptococcus mutans, fluoride and chlorhexidine varnish, biofilm.

Introduction

Microbial biofilm is defined as the diverse community of microorganism embedded in an extracellular matrix of host and microbial polymers. Dental plaque is also a biofilm found on the tooth surface and since it possesses the properties of biofilm, it usually predisposes to dental caries[1,2]. Of the various substances and varnishes that have been tried to prevent dental caries, fluoride and chlorhexidine varnishes are on trial and the effects of these varnishes on the biofilm produced by *Streptococcus mutans* need to be determined.

Tooth decay or dental caries, is an important disease of people worldwide. It is formed through interaction between acid-producing bacteria and fermentable carbohydrates as well as many host factors[3]. *Streptococcus mutans* among all microorganisms are most closely associated with development of caries. *S. mutans* synthesises insoluble glucan and glucosyltransferase from sucrose and these substances are very essential in adhesion. Hence, any agents that can interfere with the adherence property of S. *mutans* could control dental caries[4,5].

According to Emilson CG, chlorhexidine remains the most widely studied antimicrobial agent for control of plaque formation and the effect depends on it's concentration in the varnish and number of applications on the tooth surface[6,7]. The other agent frequently used for prevention of dental caries is fluoride.

Objectives: The study aimed at finding the action of chlorhexidine varnish (CHX) and fluoride varnish (F) on formation of biofilm by *Streptococcus mutans* isolated from dental plaque.

Material and Methods

The study was a continuation of the work done to study the effect of varnish containing fluoride and chlorhexidine (CHX) on *Streptococcus mutans* isolated from dental plaque of children[8]. The study groups were as follows: Group1-following fluoride varnish treatment, Group 2-Following chlorhexidine varnish treatment, Group 3- Control group (by stratified block randomization).

The organisms were isolated from Mitis–Salivarius-Bacitracin (MSB) medium with sucrose (200gm/l) and Bacitracin (0.2 U/ml).

Colonies of *Streptococcus mutans* were preserved in brain heart infusion broth with 20% glycerol and stored at -20°C.

Biofilm Assay of *Streptococcus mutans* by Microtitre plate method by O'Toole and Kolter[9]: The organisms were grown

in brain heart infusion broth for 24h. 200µl of 1:100 diluted brain heart infusion broth cultures was inoculated into flat bottomed 96 well tissue culture plates and incubated at suitable temperature (37^0C) separately for 48h. The contents of each well was aspirated, fixed and stained with crystal violet. Then the plate was washed with water. After drying, optical density (OD) was read with micro ELISA plate reader at 570 nm. In a similar manner, the organisms grown after treatment with chlorhexidine varnish and fluoride varnish after duration of 48h, 1 week and 3 months were collected and preserved. The biofilm produced was again recorded. The results were tabulated.

Statistical analysis: The results were compiled systematically and analysed using SPSS vers.17.0. Group comparison was done by ANOVA test and intergroup comparison at different time intervals was done by Bonferroni t test. Comparison between the groups was done by Tukey's test.

Results and Discussion

There was a significant decrease in biofilm production of *Streptococcus mutans* after 48h following chlorhexidine varnish application when compared to the biofilm production of *Streptococcus mutans* isolated from the controls. However there was an increase in biofilm production in the isolates after fluoride varnish application. Plaque collected following one month after application of varnish showed that there was a significant decrease in biofilm production in isolates of *Streptococcus mutans* exposed to fluoride and chlorhexidine varnish as compared to controls. After three months, the organisms isolated showed a significant decrease in biofilm production in both the varnish groups (figure-1).

Intergroup comparison by Bonferroni t test showed that the fluoride group showed an increase in biofilm after 48h as compared to controls. There was a significant decrease in biofilm in *Strepotococcus mutans* isolated from chlorhexidine varnish treated group when compared to controls. Similarly the biofilm produced by the isolates at one month and three months in the study groups was significantly decreased compared to the controls as shown in table-1.

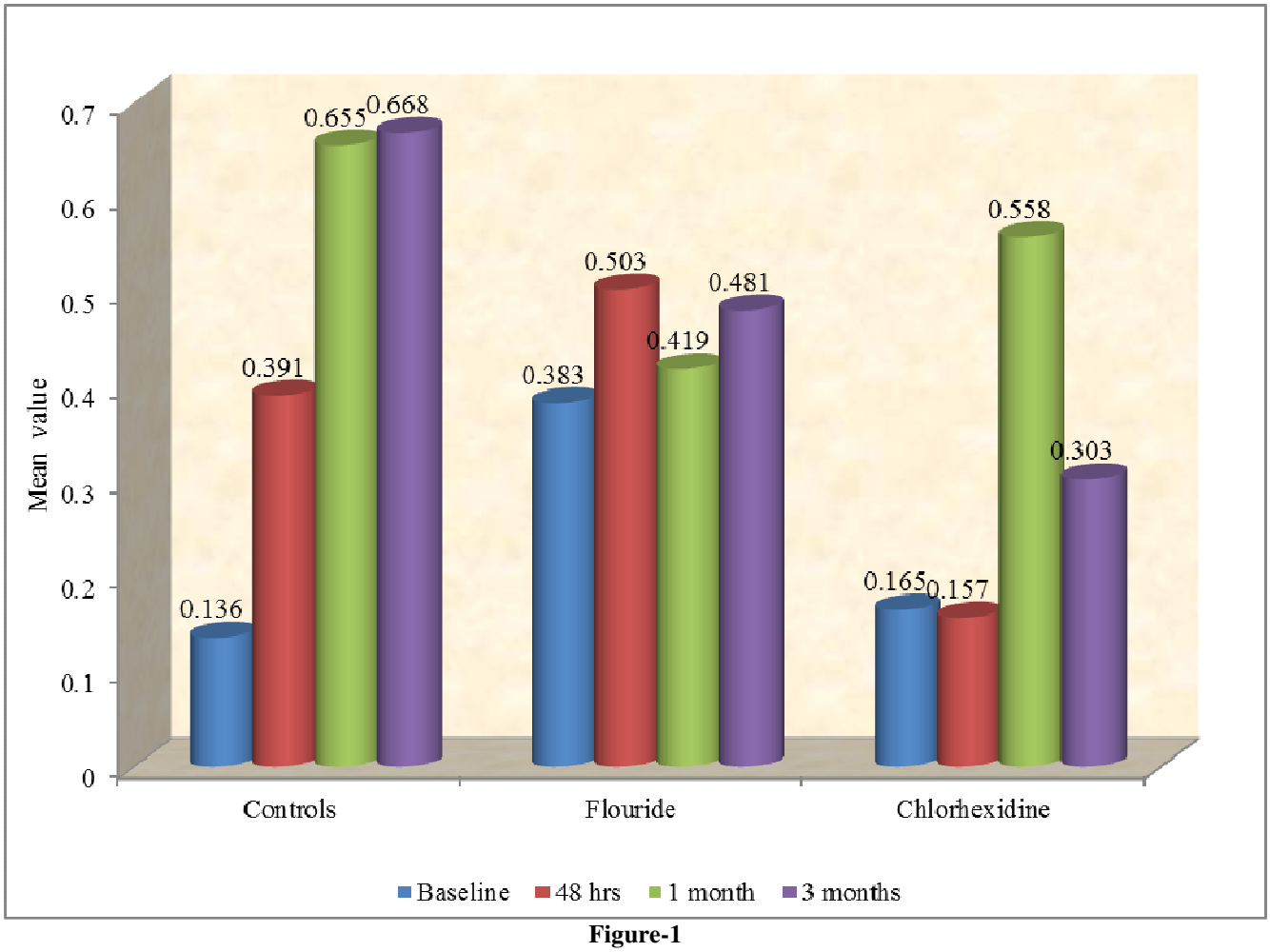

Figure-1
Comparison of biofilm production between various study groups

Table-1
OD values of the biofilm produced by the isolates

Group	Time duration	Mean OD	SD	F value	P value
Control	Baseline	0.136	0.089	19.549	< 0.001 vhs
	48 h	0.390	0.17		
	One month	0.650	0.16		
	Three months	0.668	0.14		
Fluoride varnish treated	Baseline	0.383	0.125	2.125	0.143 ns
	48h	0.503	0.18		
	One month	0.419	0.17		
	Three months	0.481	0.14		
Chlorhexidine treated	Baseline	0.165	0.112	38.347	<0.001 vhs
	48 h	0.157	0.08		
	One month	0.558	0.23		
	Three months	0.303	0.10		

NS –not significant, VHS-Very highly significant

Discussion: Various formulations have been tried to prevent the formation of biofilm on tooth surfaces and therefore to prevent dental caries. The effect of various varnishes on the microbiota, particularly on bacteria associated with caries has undergone clinical and *in vitro* trials[4-6]. The present study used fluoride and chlorhexidine varnishes. Chlorhexidine containing varnishes produce long lasting suppression of *S. mutans* while fluoride may interfere with bacterial growth and metabolism[7]. We found that although fluoride/ chlorhexidine caused suppression of biofilm forming property of *S. mutans* after 1 month of treatment, it failed to completely inhibit the growth of the organism after 3 months duration and there was a slower rate of biofilm production. The interesting feature was that more amount of biofilm was produced when compared to biofilm produced after 1 month following varnish treatment.

A process termed initial burst has been found to exist in case of some varnishes. The active agent is released rapidly and then at a slower rate[10-12]. The present study suggests that this could be the reason for the decreased biofilm production of the isolates grown after 1 month of treatment with varnish.

Our study found that as the concentration of varnish decreased, isolation rate increased. This could be due to the very low concentration of varnish remaining after the initial excessive release which may be inefficient to inhibit the formation of biofilm. An interesting finding in our study was that the isolation rate of *S. mutans* was greatly inhibited following 48 h after varnish treatment but the biofilm formation was increased. This result could be considered due to the increased release of fluoride from the varnish (burst effect).

Most bacterial species in the oral cavity have been found to be inhibited by chlorhexidine. Combination of chlorhexidine and fluoride have a greater action on phosphorus and potassium metabolism than when used alone, thereby suggesting that it could be the preferred choice for the treatment of caries[13-15].

Conclusion

This study suggests that there is a gradual loss of action of the varnish containing fluoride and chlorhexidine on viability of *Streptococcus mutans*, but it can still decrease the biofilm producing property of the organism *in vitro*.

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