EFFECT OF FLUORIDE VARNISH ON STREPTOCOCCUS MUTANS COUNT IN PLAQUE OF CARIES FREE CHILDREN USING DENTOCULT SM Strip Mutans TEST

A Randomized Controlled Triple Blind Study

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Certificate

This is to certify that **Dr. J. Jeeva Rathan**, has done this dissertation titled ***"EFFECT OF FLUORIDE VARNISH ON STREPTOCOCCUS MUTANS COUNT IN PLAQUE OF CARIES FREE CHILDREN USING DENTOCULT SM Strip Mutans TEST- A Randomized Controlled Triple Blind Study"*** under our direct guidance and supervision, in partial fulfillment of the regulations laid down by *THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY*, Chennai for the ***MASTER OF DENTAL SURGERY*** **(PEDODONTICS AND PREVENTIVE DENTISTRY)** degree examination.

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CONTENTS

INTRODUCTION

Dental caries is a carbohydrate modified local infection destructing the hard tissues of a tooth. It is a complex disease which is expressed as an interaction of various factors like host, agent, substrate and time. Most important in the understanding of caries process is that dental caries does not occur either in the absence of dental plaque or dietary fermentable carbohydrate, hence it is considered as a dietobacterial disease[11]. According to Van Houte J (1994)[78], Streptococcus mutans play a significant role in the development of dental caries where as Loesche WJ (1986)[41] said that it is the chief pathogen. Modern concepts consider caries as an interaction between genetic and environmental factors in which social, behavioral, psychological and biological factors are expressed in a highly complex interactive manner[56]. If caries has to occur and progress the above conditions should be favorable. Hence the caries process is dependant upon

a. The interaction of protective and deleterious effects of saliva and plaque.
b. The balance between cariogenic and non-cariogenic microbial population within saliva and plaque and
c. The physiochemical characteristics of enamel, dentin and cementum that make dental hydroxyapatite more or less vulnerable to acidogenic challenge[2,3,8,16,27-29,34,36,53,56,83].

Plaque is a soft, translucent and tenaciously adherent material accumulating on the surface of teeth. A number of endogenous oral microorganisms found in dental plaque are considered crucial to the initiation and progression of dental caries. They are Mutans Streptococci (Streptococcus mutans, Streptococcus sobrinus), lactobacillus species, actinomyces species, non-mutans streptococci and yeast and hence it is described as bacterial plaque. The microbial virulent traits strongly associated with caries include

a. The ability to produce acid and to sustain acid production at low pH levels that result in demineralization of calcified structure.

b. The formation and use of extra and intracellular storage polysaccharides that permit microorganisms to continuously produce acid even after dietary carbohydrates have been depleted.
c. The formation of water insoluble glucans that aid in the accumulation of mutans streptococci in plaque and modifying it's diffusion characteristics and allowing the substrate to diffuse to a deeper layer of plaque adjacent to the tooth surface.

Mutans streptococci possess all of these virulent traits supporting their role in caries process. They become pathogenic only under conditions that lead to frequent and prolonged acidification of dental plaque. Mutans streptococci and lactobacilli gain a selective advantage over other microorganisms as a result of their aciduric properties. Streptococcus mutans adapt to environmental low pH and thus increase their rate of acid production and drive the pH still lower resulting in a cariogenic plaque[42]. Longitudinal studies have shown a relative rise of Streptococcus mutans in plaque samples from tooth surfaces that become carious at a later stage[9,44,71,79]. Streptococcus mutans have been measured in saliva of children from different background and they have been found to correlate with patients caries activity levels[15,72].

Caries activity is a compound diagnosis derived from immediate past experience, lesion progression and the clinical appearance of the lesion or cavities. Caries activity is evaluated on the basis of data obtained from clinical examination and assessment of factors associated with the pathogenesis of the disease. These data regarding dental caries can be collected by traditional visual inspection and probing or by some objective detection methods which rely on the mineral changes as a basis for evaluation of caries activity and risk assessment[74]. None of these methods aim at the estimation of the chief pathogen Streptococcus mutans. Now microbial monitoring has been considered as an alternative method for evaluating current caries activity and future caries risk. According to Shi et al (1998)[64] stated that Dentocult SM kit is a reliable method for measuring the status of dental caries in preschool children and also a valuable tool in the prevention and treatment of dental

caries. In (2003)[65], they added that Dentocult SM is one caries activity test which is helpful for the diagnosis of caries and its progression based on the count of Streptococcus mutans.

As dental caries is of multifactorial etiology preventive measures usually involve a combination of dietary counseling, oral hygiene measures and fluoride application[73]. Oral hygiene measures aim at the removal of plaque from the tooth surfaces which contain Streptococcus mutans and other bacteria. Fluoride works primarily via topical mechanisms including inhibition of demineralization, enhancement of remineralisation at the crystal surfaces and inhibition of bacterial enzymes[28]. TenCate (1999)[70] reported fluoride concentration as low as 0.02-0.06 ppm has been shown to enhance remineralisation when enamel specimens were subjected to in-vitro demineralisation. Much of the research on fluoride focused on the interaction between it and the dental hard tissues with little or no attention paid to the effects of fluoride on the bacteria of plaque.

Fluoride at low concentration is bacteriostatic and at high concentration it is bactericidal. Marsh and Bradshaw (1990)[43] found that 19 ppm of fluoride in an in-vitro mixed culture study inhibited the growth of mutans streptococci. Hamilton (1990)[33] found a high fluoride concentration in the oral cavity might inhibit acid production by bacteria and may reduce the number of certain species. Songpaisan et al (1994)[67] reported children using 0.5% hydrofluoride solution showed low level of streptococcus mutans. Yoshiara A et al (2002)[81] found that the long term use of fluoride mouthrinse might contribute to reduction of mutans streptococci. This present study was planned to evaluate the effect of Fluor Protector fluoride varnish on the Streptococcus mutans count in plaque of caries free children.

AIMS AND OBJECTIVES

- To evaluate the effect of fluoride varnish on the count of Streptococcus mutans in plaque of caries free children using Dentocult SM Strip Mutans test.
- To estimate the count of Streptococcus mutans in plaque of caries free children using Dentocult SM Strip Mutans test.

REVIEW OF LITERATURE

Seppa L, **Tuutti H**, **Luoma H (1981)**[63]studied the benefit of semi-annual applications of sodium fluoride varnish (Duraphat) and silane fluoride varnish (Fluor Protector) in 11-13 year-old children with life-long exposure to fluoridated drinking water. Annual clinical and radiographic examinations were made on 67 children in the Duraphat group and 71 children in the Fluor Protector group. Fluoride varnish was applied semi-annually using the half-mouth technique. At the end of 2 years, the mean overall DMFS-increments on the control side and test side of the Duraphat group were 5.0 and 3.8 ($p < 0.01$) respectively, and of the Fluor Protector group 3.7 and 3.3 (NS). The caries reductions were 24% and 12% respectively. Since there were no differences between initial mean DMFS scores of the groups, it was assumed that lower increments in the Fluor Protector group were due to Fluoride ions crossing the midline and providing protection on the control side as well. When increments in the Duraphat control side and the Fluor Protector test side were compared, the caries reduction of Fluor Protector was 35% ($p < 0.01$). Fluoride varnishes provide additional benefit even when fluoride intake from drinking water is optimal.

Zickert I, Emilson CG (1982)[84] evaluated the effect of topical application of a fluoride containing varnish Duraphat, on the level of Streptococcus mutans in saliva and plaque of schoolchildren. Samples of saliva and pooled buccal plaque were taken before varnish application and 4, 10 and 21 days after treatment. Fluoride varnish treatment with or without a preceding dental prophylaxis had no significant effect on the plaque and salivary levels of Streptococcus mutans. The findings suggest that the caries-reducing effect of fluoride varnish cannot be explained by an alteration of the incidence of Streptococcus mutans in dental plaque or in saliva.

Brown LR, **White JO**, **Horton IM**, **Dreizen S**, **Streckfuss JL (1983)**[12] found that twelve consecutive wk of daily five-minute topical applications of 1% Sodium fluoride gel by non-cancer control subjects did not significantly

affect plaque concentrations of Streptococcus mutans or Lactobacillus species. Plaque fluoride levels increased 150% (P less than .001), while production of acetate and lactate decreased 40% (P less than .007) and 66% (P less than .001), respectively. Long-term (12 wk to more than five yr) fluoride gel use by post-irradiation xerostomic cancer patients was associated with increases in plaque fluoride and decreases in acidogenesis similar to those observed in the control subjects. Plaque concentrations of cariogenic organisms increased during the first yr of radiation-induced xerostomia and fluoride gel use, before starting to decline. Although sustained fluoride treatment increased (P less than .001) the ratio of fluoride-resistant to fluoride-sensitive strains, the number of patients harboring detectable S. mutans was diminished (P less than .001).

de Bruyn H, Buskes JA, **Arends J (1986)**[21] did an in vitro study on the inhibiting effect of a 24 hours application of a fluoridated varnish with various fluoride contents on demineralization of human sound enamel. The varnishes used had the same polyurethane base (Fluor Protector) and contained 0.7; 0.1; 0.05 and 0wt% fluoride respectively. A constant composition technique was used to demineralize, varnished and non-varnished specimens at a pH of 5 for 2 weeks. Microhardness measurements were carried out longitudinally after several time intervals to follow mineral loss. At the end of each experimental run, microradiography was carried out to investigate 1) lesion type, 2) lesion depth and 3) mineral loss. It is shown in this study that the fluoride releasing varnishes applied on the enamel for 24 hours can inhibit demineralization completely. No demineralization inhibition with the 0% fluoride varnish application was observed.

Burt BA, Eklund SA, Loesche WJ (1986)[13] assessed caries experience and Streptococcus mutans proportions from fissure plaque in school-children who lived at least three years during the study in a non-fluoridated community (0.2 mg/L). Residence histories permitted division of the cohort into those who had lived all their lives in non-fluoridated communities and those who had lived for some time previously in a fluoridated community. The children were aged 6-7 years at the beginning of the three-year study. Children with previous

residence in the fluoridated communities developed 26.8% less caries in their permanent teeth during the study than did the children who had lived in non-fluoridated communities all their lives (p = 0.04) and had 29.8% less caries after three years (p = 0.02). Differences between the groups in Streptococcus mutans proportions from fissure plaque, sampled at six-monthly intervals throughout the study, could not be demonstrated. Thus there was no difference in distribution of children with mutans streptococci between water-fluoridated and non-fluoridated areas.

de Bruyn H, **Buskes JA**, **Jongebloed W**, **Arends J (1988)**[20] studied the relationship between the amount of fluoride acquired by human enamel after varnish application and the resulting inhibition of demineralization. Intact human enamel were pretreated with Fluor Protector varnishes with differing fluoride contents (0.7; 0.1; 0.05 and 0 wt% F-) for 24 hours. In a first experiment the amount of fluoride acquired after application was determined. In a second experiment the pretreated enamel was stored intra-orally under constant plaque coverage, in order to create a substantial demineralization challenge. The protection against demineralization, induced by the various varnishes was determined four months after varnish application using microradiography. Under the cariogenic conditions created in this study, the fluoride containing varnishes induced a protection of 53-75%. Although the amount of fluoride uptake was strongly related to the fluoride content in the varnishes, no statistically significant difference in demineralization inhibition between the varnishes was observed. Scanning electron microscope investigation of the enamel lesions revealed globular precipitates inside the fluoridated enamel, presumably consisting of calcium fluoride-like material. The present study indicates that the fluoride content in Fluor Protector varnishes can be decreased without reducing its ability to inhibit demineralization.

de Bruyn H, **Buskes H (1988)**[19] evaluated 8 patients, who carried 3 enamel specimens (Fluor Protector, Duraphat, Control) intra-orally during 4 months. They kept plaque accumulation intact on the specimen and avoided

fluoride administration from other sources. After 4 months of substantial cariogenic challenge, the enamel was analyzed by microradiography and the degree of caries protection obtained for each varnish type was calculated. The results show that under high-risk caries conditions enamel treated with Fluor Protector was significantly better protected (65%) than enamel treated with Duraphat(3%).

Hamilton JR (1990)[33] studied the biochemical effects of fluoride on oral bacteria. Early studies demonstrated that the fluoride induced reduction in acid production was due, in part, to the inhibition of the glycolytic enzyme, enolase, which converts 2-P-glycerate to P-enolpyruvate. The decreased output of PEP in the presence of fluoride in turn, resulted in the inhibition of sugar transport via the PEP phosphotransferase system (PTS). Bacterial accumulation of fluoride involves the transport of HF, a process requiring a transmembrane pH difference or pH gradient, which is generated only by metabolically active cells. The uptake of HF into the more alkaline cytoplasm results in the dissociation of HF to H^+ and F^- and, if allowed to continue, the accumulation of protons acidifies the cytoplasm, caused a reduction in both the proton gradient and enzyme activity. Current information indicated that in addition to enolase, fluoride also inhibits the membrane-bound, proton-pumping H^+/ATPase, which is involved in the generation of proton gradients through the efflux of protons from the cell at the expense of ATP. Thus, fluoride has the dual action of dissipating proton gradients and preventing their generation through its action on H^+/ATPase. The collapse of transmembrane proton gradient, in turn, reduced the ability of cells to transport solutes via mechanisms involving proton motive force. Thus a high fluoride concentration in the oral cavity might inhibit acid production by bacteria and may reduce the numbers of certain species.

Marsh PD, Bradshaw DJ (1990)[43] in vitro model system studied the combined influences of fermentable carbohydrate, pH, and fluoride on the stability of complex oral microbial communities. The pH generated from carbohydrate pulses rather than the availability of substrate per se was

responsible for the enrichment of the cariogenic species Streptococcus mutans and Lactobacilli casei. The addition of sub-minimal inhibitory concentration level of sodium fluoride (1 mmol/L; 19 ppm) reduced both the rate of acid production and the fall in terminal pH from glucose pulses, thereby enabling pH-sensitive bacteria, including many Gram-negative species, to persist. Furthermore, the combination of even a moderately-low environmental pH (ca. pH 5.0) with a low level (1 mmol/L) of fluoride was able to prevent Streptococcus mutans from out-competing with other species and resulted in lows proportions within the bacterial community. By this mechanism, fluoride could make a significant contribution to preventing dental caries.

Adriaens ML, **Dermaut LR**, **Verbeeck RM (1990)**[1] determined whether Fluor Protector applied to molars before orthodontic banding could prevent white spot formation. In this in-vitro study, 93 human premolars were used, divided into five different groups, representing different clinical situations. Each tooth was sliced in half, one as a control and the other as a test specimen. All tooth halves were stored in a demineralizing solution, in an attempt to induce white spot formation. In the in vivo study 104 molars (52 controls and 52 tests) of 28 orthodontic patients were involved. The 'split-mouth technique' was used. After evaluation of the results of both studies, it is evident that Fluor Protector is very effective in the prevention of white spot formation under molarbands.

Berg JH, Farrell JE, Brown LR. (1990)[7] evaluated the effects of glass ionomer/silver cermet restorations on the plaque levels of interproximal mutans streptococci. Fifteen patients with Class II lesions in primary molars were selected for study. Interproximal plaque samples were obtained from each of the lesion sites and from one caries-free site approximal to a primary molar. One lesion was restored with composite resin to serve as a treated control to the glass ionomer/silver cermet (Ketac Silver, ESPE/Premier Sales Corp., Norristown, Pennsylvania) test site. A sound (unaltered) interproximal site served as the untreated control site. Plaque samples were collected before and at one week, one month, and three months post-treatment. Samples were

serially diluted to enable colony counts of mutans streptococci. One week post-treatment counts showed that the glass ionomer/silver cermet restorations significantly reduced ($p < 0.05$) the approximal plaque levels of mutans streptococci. Conversely, the untreated and treated control sites did not exhibit reductions in approximal plaque levels of mutans streptococci. These results indicate that glass ionomer restorations may be inhibitory to the growth of mutans streptococci in dental plaque approximal to this restorative material in the primary dentition.

Forss H, Jokinen J, Spets-Happonen S, Seppa L, Luoma H (1991)[31] compared the levels of fluoride and mutans streptococci in plaque grown on glass ionomer (Ketac-Fil) and composite (Silar) restorations. From tunnels left under the brackets bonded either with glass ionomer or composite, 14-day-old plaque samples were collected 14, 28, and 42 days after bonding. For glass ionomer the mean counts of mutans streptococci in plaque were 0.5×10^3, 6.7×10^3, and 8.8×10^3 CFU at the first, second, and third collection, respectively, whereas for composite restorations the corresponding values were 32.1×10^3, 14.6×10^3, and 120.6×10^3 CFU. For glass ionomer the mean concentrations of fluoride were 19,985, 5,788, and 5,019 ppm at first, second, and third collections of 14-day-old plaque samples, respectively, whereas for composite restorations the mean concentrations of fluoride were about 200 ppm throughout the study. The results showed that the fluoride level in plaque growing on glass ionomer was much higher than that on composite restorations which seems to affect the level of mutans streptococci in dental plaque.

Petersson LG, Birkhed D, Gleerup A, Johansson, Jonssom G (1991)[48] comapred the caries-inhibiting effect of four different toothpastes in a 3-year clinical and microbiological study. The paste used are 0.8% sodium monofluorophosphate (MFP) with 3% xylitol and 6% sorbitol, 0.03% sodium fluoride with 3% xylitol and 6% sorbitol, 0.8% MFP with 9% sorbitol and 0.03% sodium fluoride with 9% sorbitol. There were 284 children, aged 12-13 years old at baseline, took part in the study. After 3 years, no statistically significant differences were found between the different toothpaste groups

concerning either development of initial or gross caries lesions or number of mutans streptococci and lactobacilli in saliva.

el-Nadeef M, **Kalfas S**, **Edwardsson S**, **Ericson D (1992)**[22] studied about the discoloration of broth and colonies of mutans streptococci on the plastic strip. In an attempt to explain the above phenomenon and to investigate the influence of the salivary flora on the "Strip mutans" method, a total of 46 subjects were sampled. Saliva was analyzed using the "Strip mutans" method and conventional plating techniques to identify mutans streptococci, enterococci, staphylococci, enteric bacteria, and yeasts. Approximately 85% of the "Strip mutans" scores coincided with the conventional MSB-plating method. Two samples showed discolored mutans streptococci colonies on the "Strip mutans" strip. Enterococcus species were present in the saliva of these test subjects and could grow in the "Strip mutans" broth. Enterococcus faecalis was able to induce the same type of discoloration under experimental pure culture conditions. Three "Strip mutans" samples showed small colonies of mutans streptococci, visible only under magnification. Staphylococcus epidermidis was present in these saliva samples and showed heavy growth in the broth. Under experimental pure culture conditions Streptococcus epidermidis also inhibited the growth of mutans streptococci to some extent.

Davenport ES, Day S, Hardie JM, Smith JM (1992)[17] compared commercial kits and conventional methods for enumeration of salivary mutans streptococci and lactobacilli. Mutans streptococci (MS) and lactobacilli levels were determined by conventional and commercial dip-slide methods in three groups of young subjects, aged 5-6 years (93 subjects), 12-13 years (78 subjects) and 18-20 years (81 subjects). Using the same paraffin-stimulated saliva samples, mutans streptococci and lactobacilli were estimated by conventional viable counts on modified mitis-salivarius bacitracin agar (MSB) and Rogosa agar plates, and by inoculation of Dentocult SM and Dentocult LB dip-slides (Orion Diagnostica, Finland). The salivary mutans streptococci and lactobacilli counts obtained from conventional agar plates were significantly correlated ($P < 0.0001$) with the dip-slide estimates of these organisms. These

dip-slide tests provided suitable and simple methods for screening salivary lactobacilli and mutans streptococci levels, which may have a useful role in the assessment of caries risk.

Songpaisan Y, Sernirach R, Kuvaranasuchati J, Bratthal D (1994)[67] evaluated the level of mutans streptococci in two groups of children Thai (Bangkok) to relate the caries prevalence and the caries increment over 2 years and to study whether different sealant and fluoride programs affected levels of mutans streptococci over a 2-year period. The baseline survey comprised 1,114 children aged 12 years. For the sealant project, a minimum of three caries-free permanent molars was required; 752 children aged 12-13 and 512 children aged 7-8 years were distributed into five groups: control group, Delton fissure sealant group, glass ionomer fissure sealant applied by dentist (GIC-dentist group) or by school teachers given a 3-day course (GIC-teacher group), and an HF group (0.5% HF solution applied 3 times). The WHO standard criteria were used to record caries. Prevalence of mutans streptococci was estimated using the strip mutans test. 17% were in class 0 (low level), 32% in class 1, 33% in class 2 and 18% in class 3. The corresponding mean DFT +/- SD for each mutans streptococci class was 1.84 +/- 2.33, 2.23 +/- 2.14, 3.18 +/- 2.75, and 3.59 +/- 3.01 respectively. For the 7- to 8-year-olds (n = 512), mean df teeth at baseline was 5.36 (d = 5.19; f = 0.17) and 5% were in class 0, 17% in class 1, 33% in class 2 and 45% in class 3. The corresponding mean df teeth was 3.19 +/- 2.5, 4.13 +/- 2.84, 4.89 +/- 2.94 and 6.39 +/- 3.16 respectively. They concluded that the children who received the HF solution had lower levels of mutans streptococci.

Banoczy J, Gombika A, Szoke J, Nasz I (1995)[6] evaluated the effect of the simultaneous application of a chlorhexidine and thymol-containing varnish (Cervitec) and an amine fluoride/stannous fluoride containing toothpaste (Meridol) on Streptococcus mutans counts in saliva and dental plaque of school children 12-14 years of age, during a six-week period. The children were separated into group I (Cervitec varnish + fluoride-containing toothpaste), group II (Cervitec varnish + Meridol toothpaste), and group III

(Meridol toothpaste alone). Over the six weeks the greatest improvement in salivary Streptococcus mutans count occurred in group II. Overall, a statistically significant decrease in total microbiological count and Streptococcus mutans was found in all three groups.

Schlagenhauf U, **Pommerencke K**, **Weiger R (1995)**[58] studied the influence of toothbrushing, eating and smoking on the reliability of Dentocult SM test scores in 30 subjects aged 21-39 years. All experiments were performed 24 hours after professional tooth cleaning in the morning of 3 consecutive days. On day 1 immediately before and 30 min after toothbrushing, Dentocult SM tests were taken and total salivary colony-forming units per ml were determined. Following the same protocol, the influence of a standardized breakfast and of smoking was evaluated on day 2 and on day 3 respectively. Although all parameters significantly decreased the salivary colony-forming units per ml, only the standardized breakfast induced significant changes in the Dentocult SM test results. Eating therefore should be avoided prior to the performance of the Dentocult SM test.

Pienihakkinen K, **Jokela J (1995)**[52] assessed the practicability of the Dentocult SM test in children, using dental floss to transfer the dental plaque to the strip. The subjects were children of 2-3 yr (n = 365) and 5-6 yr (n = 398). The mutans streptococci count on the strip was found to be a good indicator of infection and was surprisingly accurate in the prediction of the 3 year caries increment.

Twetman S, Petersson LG, Pakhomov GN (1996)[75] studied the caries incidence in relation to salivary mutans streptococci and fluoride varnish applications in preschool children from low and optimal fluoride areas. Caries incidence during a 2-year period was studied in 4- to 5-year-old children from three areas with contrasting levels of natural fluoride (F) in the drinking water and different regimens of topical fluoride varnish applications; group A (n = 448) was from an area with a low level of F (0.1 ppm) and semi-annual applications of fluoride varnish; group B (n = 374) was from a low F area (0.1 ppm) and no fluoride varnish treatments; group C (n = 206) was from an area

with optimal F (1.2 ppm) and fluoride varnish treatments. All children were clinically assessed at baseline and after 2 years according to World Health Organization criteria. The number of salivary mutans streptococci was estimated and scored at baseline and after 2 years with the strip mutans method. The varnish containing 0.1% F was applied every 6 months on all accessible tooth surfaces after cleaning with a pumice paste. Higher levels ($p < 0.05$) of salivary mutans streptococci were found in the low-fluoride areas compared to the optimal fluoride area at baseline and after 2 years. The caries incidence (mean dft +/- SD) in the different groups was A: 0.65 +/- 1.40; B: 1.09 +/- 1.85; C: 0.53 +/- 1.09. The difference between group B and groups A and C was statistically significant ($p < 0.05$). A positive relationship ($p < 0.05$-0.001) between salivary mutans streptococci scores at baseline and caries incidence was found in all three groups. This study confirmed the close association between salivary mutans streptococci and caries incidence in preschool children and suggests a caries-reducing effect of topical applications of the fluoride silane varnish.

Seppa L, Hauseen H, Karkkainen S (1996)[60] compared plaque fluoride and the level of mutans streptococci in saliva and plaque before and 1 and 2 years after discontinuation of water fluoridation in Kuopio, Finland. For comparison, a low-fluoride community was included in the study. Pooled plaque and saliva were collected from a random sample of 12-year-olds in both communities (n = 139). Enumeration of mutans streptococci in plaque was made on MSB agar and the level of salivary mutans streptococci was measured using the strip mutans method. Fluoride was analyzed using a fluoride specific electrode. Caries, gingival status, fluoride varnish applications and self-reported oral health habits were recorded at baseline. Before discontinuation of fluoridation, the level of mutans streptococci in saliva was significantly lower in the fluoridated than in the non-fluoridated community. The difference in plaque mutans streptococci was not statistically significant. After discontinuation of water fluoridation, there was a significant shift towards elevated values of salivary mutans streptococci in the fluoridated community,

but the level of mutans streptococci in plaque remained at the baseline level. There was no significant difference between the communities in the fluoride content of plaque either before or after discontinuation of fluoridation. From the background factors, only caries scores (higher in the non-fluoridated community) and oral hygiene (better in the non-fluoridated community) were significantly different between the communities.

Ogaard B, **Larsson E**, **Glans R**, **Henriksson T**, **Birkhed D (1997)**[47] studied the effect of combined application of a fluoride varnish (Fluor Protector) and an antimicrobial varnish (Cervitec) on the oral microflora, caries and gingival condition in patients receiving treatment with fixed orthodontic appliances. A total of 198 individuals (12 to 15 years old), scheduled for fixed orthodontic treatment, were randomized into 2 groups. Prior to bonding, the Cervitec and the control group received one application with Cervitec or a placebo every week for 3 weeks, respectively. In the Cervitec group Fluor Protector was applied at bonding and Cervitec at the next visit, 6 weeks later. Each varnish was then applied every 12 weeks for 24 weeks. In the control group, the fluoride varnish was applied only at bonding and every 12 weeks. The Visible Plaque Index (VPI), the Gingival Bleeding Index (GBI), the White Spot Lesion Index (WSL) and the level of mutans streptococci in plaque and saliva were recorded 3 weeks prior to bonding and after 24 weeks. At bonding and after 12 weeks, only VPI, GPI, plaque and salivary mutans streptococcus counts were recorded. During the 3-week prebonding period, the mean VPI, GBI and mutans streptococci in plaque decreased in both groups. At bonding, the mean level of mutans streptococci in plaque was significantly lower in the Cervitec group than in the control group. The mean level of mutans streptococci was significantly lower after 12 weeks' bonding in the Cervitec group than in the control group. No effects on the other parameters were found during the 24 weeks.

Arends J, **Duschner H**, **Ruben JL (1997)**[4] evaluated three different varnishes employed in caries prevention (Duraphat, Fluor Protector and Cervitec) into demineralized dentin using confocal laser scanning microscopy.

The results showed that the varnish penetration into lesions about 85 microns in depth for Cervitec about 35 microns and considerably less for Duraphat and Fluor Protector. The penetration was into the dentinal tubules and it was influenced by dentinal tubule direction. The drying procedure, pretreatment of the dentin influences the penetration, only for Cervitec applications. This paper showed that varnish penetration into the tissue and presumably 'sealing' tubules completely or partly is valuable with respect to root caries prevention and hypersensitivity.

Eronat C, Alpoz AR (1997)[26] assessed the efficiency of a 1% chlorhexidine-containing varnish (Cervitec, Vivadent, Liechtenstein) on the levels of Streptococcus mutans in saliva of patients with fixed orthodontic appliances using the Dentocult SM (Vivadent, Liechtenstein) technique for the microbiological investigation. Eighty subjects participated in the study and divided randomly into two equal groups in which one group was treated with the placebo varnish (Vivadent, Liechtenstein) for negative controls. Streptococcus mutans in saliva of the subject was sampled and enumerated by using the Dentocult SM dip-slide technique for periods of one, two, four and twelve weeks after a single varnish application. The results were evaluated statistically. After the chlorhexidine containing varnish treatment, the levels of Streptococcus mutans in saliva were significantly reduced after one week ($p < 0.01$) and continued reduction for one month ($p < 0.05$). After twelve weeks Streptococcus mutans levels in saliva were given a relative increase. No significant suppression was found in the placebo group ($p > 0.05$). The results suggested that Cervitec varnish reduces salivary Streptococcus mutans levels and that the application should be repeated every 3 months to get antibacterial effect.

Twetman S, Petersson LG (1997)[76] evaluated and compared the efficacy of a chlorhexidine/ thymol- containing (CHX) and chlorhexidine/ thymol/ fluoride-containing (CHX + F) varnish to decrease interdental levels of mutans streptococci. Eighty-two healthy schoolchildren (11-13 years) with high scores of salivary MS were selected by a screening procedure and

randomized into two groups. Mutans streptococci were enumerated at all mesial interdental sites of the first permanent molars with the aid of a modified chair-side technique. The interdental molar and premolar sites were treated with either a 1% chlorhexidine/thymol varnish (Cervitec) or a 1:1 mixture of the chlorhexidine/thymol varnish and a fluoride varnish containing 0.1% difluorsilane (Fluor Protector; chlorhexidine/thymol/fluoride-containing) on two occasions within a 2-week period. The varnishes were applied with a small brush after cleaning with dental floss and drying with air. Follow-up samples from the interdental areas were collected after 1 and 3 months. Both groups exhibited a similar statistically significant ($p < 0.05$) reduction of interdental mutans streptococci after 1 month when compared with baseline. After 3 months, a significant reduction ($p < 0.05$) was still found in the chlorhexidine/thymol/fluoride containing varnish group but not in the chlorhexidine/thymol varnish group. In conclusion, the results suggest that the addition of fluoride to an antibacterial varnish might improve the long-term efficacy in diminishing the cariogenic microbial challenge. Thus, the mixed varnish concept should be further developed and warrants an implementation of clinical studies.

Shi S, Liang Q, Yakushiji M, Machida Y (1998)[64] studied the relationship between caries activity and the status of dental caries in preschool children using a caries activity test (CAT). The status of primary tooth caries in 229 children ages 3 to 5 was examined. Caries incidence, dft, and caries severity index (CSI) were calculated. Based on the quantity of Streptococcus mutans in the oral cavity detected with Dentocult SM, a caries activity test, four grades of caries activity were classified: Grade 0 ($< 10^4$ colony-forming units/ml), Grade I (10^4-10^5 CFU/ml), Grade II (10^5-10^6 CFU/ml), and Grade III ($> 10^6$ CFU/ml). The results showed that 79.48% children had Grade I or higher caries severity. Significant differences of caries activity were found among different grades, which were highly correlated with caries incidence, dft, and caries severity index ($r = 0.22216$, 0.31212 and 0.32276 respectively). This study concluded that Dentocult SM is a reliable method for measuring the

status of dental caries in preschool children. It is also a valuable tool in the prevention and treatment of dental caries.

Petersson LG, **Twetman S**, **Pakhomov GN (1998)**[51] studied the caries inhibitory effects of semiannual applications of a fluoride varnish in preschool children. Twenty-four public dental health clinics in the county of Halland, Sweden, with 5,137 preschool children, 4 and 5 years of age, were matched and equally allocated to a fluoride varnish group (n = 2,535) and a reference group (n = 2,602). The children in the fluoride varnish group were treated every six months with topical applications of a silane fluoride varnish, Fluor Protector (0.1% F) while no fluoride varnish was used in the reference group. Both groups received a basic preventive program at annual checkups consisting of dietary counseling and instructions to parents to brush their children's teeth at least once daily with fluoridated dentifrice. Caries data were collected by clinical examinations at baseline and after one and two years. Caries prevalence at baseline did not differ significantly between the groups. After two years, the mean caries incidence was low and no statistical difference was found in the total number of carious and filled surfaces (dfs) between the two groups. However, the incidence of approximal lesions (dfsa) was significantly lower ($P < .05$) in the fluoride varnish group than the reference group. Children in the fluoride varnish group with dfs scores of 1-4 and ≥5 at the start of the study exhibited a statistically significant ($P < .05$) reduction in approximal caries incidence of 19 percent and 25 percent, respectively, when compared with the reference group. Thus preschool children of 4 and 5 years of age with clinical caries who receive semiannual applications of a silane fluoride varnish containing 0.1 percent F experience a reduced incidence of approximal caries over two years.

Petersson LG, **Magnusson K**, **Andersson H**, **Deierborg G**, **Twetman S (1998)**[50] evaluated the progression of approximal caries in a group of 115, 12-yr old children, who were treated semi-annually with a mixture (1:1) of a varnish containing 0.1% F (Fluor Protector) and 1.0% chlorhexidine (Cervitec). A reference group of 104 children received fluoride varnish

treatment (Fluor Protector) semi-annually. Approximal caries was recorded from bitewing radiographs at baseline and after 3 years. At baseline, decayed and filled surfaces (DFS) including enamel caries were 1.79+/-2.36 in the reference group and 2.0+/-2.77 in the test group. After 3 yr, the mean approximal caries incidence including enamel caries was 3.01+/-3.74 and 3.78+/-4.32, respectively. The differences at baseline as well as after 3 yr were not statistically significant. The results showed that both groups had a comparatively low incidence of approximal caries during the experimental period, and suggest that a mixture of fluoride and antibacterial varnish had no additional preventive effect on approximal caries incidence compared with fluoride varnish treatments alone.

Twetman S, **Skold-Larsson K**, **Modeer T (1999)**[77] studied the fluoride concentration in whole saliva and in separate gland secretions after a single application of each of 3 different fluoride varnishes with contrasting levels of fluoride in a randomized crossover design. The study group comprised 8 healthy schoolchildren aged 10-12 years treated with A: Bifluorid 12 (6% F), B: Duraphat (2.26% F) and C: Fluor Protector (0.1% F). Unstimulated and stimulated whole saliva, as well as stimulated parotid and submandibular-sublingual saliva, were collected at baseline and at 1, 6, 12, and 24 hours after the varnish treatments. The fluoride concentrations were determined with an ion-selective electrode. Time and dose dependent concentration curves were obtained in all the collected secretions which was in the order of A > B > C. In whole saliva, the fluoride levels were significantly elevated ($P<0.01$) 1 hour after the A and B varnish applications compared with baseline, while the increase was insignificant for varnish C. Similar patterns were unveiled in the parotid and submandibular-sublingual secretions, although the increase in fluoride concentration was modest. The elevated levels did not exceed 6 hours for any of the varnish tested. The results of this study suggest a correlation between the concentration of fluoride of the varnish and fluoride levels obtained in saliva after application.

Splieth C, Bernhardt O (1999)[68] evaluated the validity of a site-specific chair-side mutans streptococci (MS) test for the prediction of caries incidence in fissures. In 230 6- to 7-yr-old children, occlusal plaque samples of teeth 16 and 36 were cultured with Dentocult SM tests at 37 degrees Celsius for 24 hours. Caries (DMFS), initial caries, sealants, and a plaque index (QHI) were recorded and oral hygiene habits were assessed. Not erupted, carious, filled and sealed teeth were excluded from the analysis (n = 154). After 2 yr, the status of the fissures was re-examined, and a fluoride history was recorded with a questionnaire filled out by the children's parents. Sealed teeth were excluded again (n = 54). With a classification of MS score 0 or 1 as low and MS score 2 or 3 as high caries risk, 92% agreement was reached by two independently working examiners. The MS scores and caries incidence correlated significantly. Seventy-eight % of the caries progression in fissures was prognosed correctly. Sensitivity was 50%, specificity 82%, positive predictive value 29%, and negative predictive value 92%. Children with caries progression tend to have lower fluoride scores. Low MS scores were most likely to be associated with low caries incidence, while high mutans streptococci scores seem to be partially compensated by other parameters.

Llena-Puy MC, Montanana-Llorens C, Forner-Navarro L (2000)[40] evaluated the cariogenic bacteria and its relation to dental caries. Age of subject, a history of caries affecting the primary dentition, the prevalence of Streptococcus mutans, pH values, salivary flow, and the frequency and amount of sugar consumption have been the factors most studied. A cross-sectional study was made of schoolchildren in the 12-13 year age range to evaluate the relationship between dental caries and colony forming units/ml of Streptococcus mutans and Lactobacillus, salivary buffer capacity, and salivary flow. Likewise, an evaluation is made of the predictive value of the variables, bacterial count and salivary pH with respect to caries. A total of 167 children were subjected to oral examination to establish the DMFT and DMFS indices, followed by the collection of saliva for quantitating Streptococcus mutans, Lactobacillus, pH and salivary flow by the Dentocult SM, Dentocult LB and

Dentobuff systems (Vivadent). Statistically significant ($p < 0.001$) correlations were observed between the caries indices and bacterial counts. No significant association was recorded with the rest of the variables studied. Bacterial counts as well as salivary buffer capacities exhibited greater negative than positive predictive values, i.e., they were more effective in identifying healthy individuals than patients who required treatment.

Petersson LG, **Magnusson K**, **Andersson H**, **Almquist B**, **Twetman S (2000)**[49] compared the effect of two different dental varnishes on approximal caries incidence in teenagers with proven caries susceptibility during a 3-year period. Two hundred 13 to 14 year old subjects exhibiting at least two approximal enamel caries lesions were selected to take part in the study. One hundred and eighty subjects participated after informed consent and were randomly assigned to two equally sized groups. One group was treated with a fluoride varnish (FV, Fluor Protector) containing 0.1% F every 3rd month and the participants of the other group were treated in the same mode with a chlorhexidine varnish (CV, Cervitec (R) containing 1% chlorhexidine and 1% thymol. In total, each subject was treated 12 times during the experimental period. Approximal caries including enamel lesions (DMFS) were recorded from four bitewing radiographs exposed at the start and end of the study. The mean (+/-SD) caries prevalence at baseline was 2.2+/-3.4 in the fluoride varnish group and 2.5+/-4.0 in the chlorhexidine varnish group. After 3 years, the average approximal caries incidence was 2.7+/-3.1 and 3.1+/-3.5 in the fluoride varnish and chlorhexidine varnish groups respectively. The differences at baseline and after 3 years were not statistically significant. In conclusion, treatments every third month with a fluoride or a chlorhexidine/thymol-containing varnish showed a promising effect with low approximal caries incidence and progression in teenagers with proven caries susceptibility.

Zaura-Arite E, **ten Cate JM (2000)**[82] compared the effects of Cervitec, containing 1% chlorhexidine (CHX) and 1% thymol, Fluor Protector, containing 0.1% fluoride, their 1:1 mixture, and a placebo varnish on the

percentage of mutans streptococci and lactobacilli in plaque and on the underlying dentin demineralization, as assessed by microradiography. Bovine dentine discs, fitted with three parallel grooves, received one of the varnish treatments into the first groove and on the adjacent part of the dentin surface. Volunteers (n = 23) wore the discs fixed to their partial dentures for four consecutive 3 wk periods. Microbiological analysis of plaque accumulated in the grooves showed no difference between groups. Fluoride varnishes (Fluor Protector and mixed varnish) had a significantly larger inhibitory effect on mineral loss in the treated groove than Cervitec or placebo. All treatment varnishes had more pronounced effect in panelists (n = 14) with higher degree of demineralization. In these panelists, chlorhexidine containing varnishes showed an inhibitory effect on demineralization in all grooves, also in the two non-varnished grooves. As fluoride varnishes had the largest localized effect on demineralization and chlorhexidine varnishes were showing a peripheral effect, a combined treatment could be the preferred method to obtain an optimal caries preventive effect in caries-prone individuals.

Skold-Larsson K, Modéer T , Twetman S (2000)[66] studied the fluoride (F) concentration in plaque after a single topical application of different fluoride varnishes with contrasting levels of fluoride. Thirty adolescents (12-17 years) with fixed orthodontic appliances were randomly assigned to one of three groups: Bifluorid (6% F), Duraphat (2.23% F) and Fluor Protector (0.1% F). The varnishes were applied after professional cleaning in one upper quadrant, leaving the opposite quadrant untreated according to the split-mouth technique. Pooled plaque samples from each quadrant were collected at baseline, 3 days, 7 days and 30 days after the varnish treatment, and fluoride was analysed by microdiffusion. All fluoride varnishes increased the fluoride concentration in plaque compared with baseline, and the mean values varied between 23 and 138ng F/mg after 3 days, depending on varnish fluoride concentration. Compared with the control quadrant, statistically significant elevations were recorded for Bifluorid after 3 days and 7 days and Duraphat after 3 days, while no significant differences

were revealed in the Fluor Protector group. The fluoride concentration in plaque was back to baseline levels for all participants in the Duraphat group after 7 days, while some individuals in the Bifluorid and Fluor Protector groups still registered slightly increased levels after 30 days. The results suggest that fluoride varnish treatments resulted in elevated fluoride levels in plaque adjacent to fixed orthodontic appliances for a period of up to 1 week, although different patterns was disclosed for the various brands.

Ekenbäck SB, Linder LE, Lönnies H (2000)[23] evaluated the effect of four different dental varnishes on the colonization of mutans streptococci, total streptococci and lactobacilli on exposed sound root surfaces. Sixty-five individuals were randomly allotted to one of four groups for treatment with Cervitec((R)) varnish containing 1% chlorhexidine and 1% thymol, a thymol varnish or one of two different fluoride varnishes, Fluor Protector and Duraphat. The varnish was applied to three buccal root surfaces in each patient at baseline and after 1 week. Dental plaque from the root surfaces was collected and analysed on four different occasions: at baseline, after 1 week, 1 month and 6 months. The Cervitec varnish caused a statistically significant reduction in the number of mutans streptococci over time. The reduction was significant at 1 week and 1 month relative to baseline. The numbers of total streptococci and lactobacilli were not significantly affected by treatment with Cervitec. No statistically significant difference over time was found for mutans streptococci, lactobacilli or total streptococci after treatment with the fluoride varnishes or the thymol varnish.

Ekenbäck SB, Linder LE, Sund ML, Lönnies H (2001)[24] studied the effect of high fluoride concentrations on carbohydrate metabolism in Streptococcus mutans present in biofilms on hydroxyapatite and the effect of fluoride-bound hydroxyapatite on lactic acid formation in growing biofilms of streptococcus mutans. Biofilms of a clinical strain of Streptococcus mutans on saliva-coated hydroxyapatite beads were incubated with sodium fluoride over a wide range of concentrations. At high fluoride concentrations (>10 mM) the incorporation of [^{14}C]-labeled glucose decreased by 80–85%, at both pH 7.0

and 5.6. At lower fluoride concentrations, the effect of fluoride on the incorporation of labeled glucose was pH-dependent in both biofilm cells and in planktonic cells. At pH 7.0, fluoride at concentrations <10 mM had little or no effect. Pretreatment of hydroxyapatite discs with fluoride varnish (Fluor Protector) or fluoride solutions caused a statistically significant reduction of lactic acid formation in associated, growing biofilms of Streptococcus mutans. Fluoride varnish and 0.2% (47.6 mM) sodium fluoride solution exhibited a statistically significant inhibitory effect on lactate production.

Munshi AK, Reddy NN, Shetty V (2001)[46] compared the caries preventive efficacy of three fluoride varnishes Fluoritop SR, Fluor Protector and Bifluorid 12. The demineralization inhibitory effects and the antibacterial effects on Streptococcus mutans were studied (in-vitro). Calcium and Phosphorus dissolutions were estimated as a measure of the demineralization inhibitory effect. Antibiotic sensitivity tests using the serial tube dilution method and disk diffusion method were used to evaluate the antibacterial effects of the fluoride varnishes. Of the three varnishes, Fluor Protector was seen to exhibit the highest demineralization inhibitory effect, while Fluoritop SR was found to be comparable to Bifluorid 12 in its caries protective effects.

Yoshihara A, Sakuma S, Kobayashi S, Miyazaki H (2001)[81] studied the effect of fluoride mouthrinse on mutans streptococci and lactobacilli in saliva. It consisted of 414 subjects aged 7, 10 and 12 years. 243 children received fluoride mouthrinse while the remaining 171 children belong to “no mouthrinse group”. The level of mutans streptococci and lactobacilli for the subjects in both the groups were measured using Dentocult SM and Dentocult LB strips. Dental examinations were done to collect data on caries prevalence. There was a significant reduction in caries between mouth rinse and no mouth rinse group in all ages. Children with fluoride mouthrinse group had lower Streptococcus mutans count than the no mouthrinse group. There was no significant relationship between the experience of fluoride mouthrinse and the score of Dentocult LB dip slide. The results suggest that the fluoride mouthrinse might contribute to reducing the count of mutans streptococci.

Seki M, Karakama F, Ozaki T, Yamashita Y (2002)[59] investigated the detection of mutans streptococci in individuals using several modifications to a commercially available kit, Dentocult SM. Significantly better detection of mutans streptococci was achieved using plaque from the four approximal surfaces at two interdental spaces than with saliva ($P < 0.001$). Furthermore, the mutans streptococci estimates for approximal surfaces at the same interdental space were similar (kappa = 0.654) suggesting that differentiating the two surfaces does not improve the detection of mutans streptococci and that increasing the number of interdental spaces sampled is a more effective option. This study also evaluated a modification to the standard Dentocult SM site strip method in which two strips were incubated per broth vial so that plaque from eight interdental spaces could be tested at the same time (new method). The results were compared to those obtained when one strip was incubated per broth vial (standard method). Although the mutans streptococci estimates by the new and standard methods were comparable (kappa = 0.721), the efficiency of mutans streptococci detection was improved significantly by increasing the number of sites used for mutans streptococci estimates ($P = 0.01$). In conclusion, mutans streptococci detection at eight interdental spaces is recommended using the new Dentocult SM method.

Autio JT (2002)[5] evaluated the effect of xylitol gum in salivary Streptococcus mutans levels in preschool children. Sixty-one children were randomly assigned into the xylitol group and the control group. The xylitol group chewed gum sweetened only with xylitol (XyliFresh100%, Hershey Food Corporation, U.S.A.) three times a day for three weeks. Streptococcus mutans counts were tested using the Dentocult SM test (Orion Diagnostica, Finland) at baseline and after three weeks. The shift from higher Streptococcus mutans scores to lower was greater in the xylitol group than in the control group ($p<0.05$). This study supports the suggestion that chewing xylitol gum may reduce salivary Streptococcus mutans levels. Xylitol chewing gum may provide a feasible caries prevention method for preschool children.

Shi S, Deng Q, Hayashi Y, Yakushiji M, Machida Y, Liang Q (2003)[65] studied the efficacy of three caries activity test's (CAT) i.e. (Dentocult SM, Dentocult LB and Dentobuff Strip) in revealing caries condition and predicting caries progress, and provide a reference for application by comparing the three tests. Oral condition and results of the three caries activity test's of 82 children aged 3 to 4 were recorded and followed up. The examination was done again two years later. The caries incidence, dft and caries severity index data from the two examinations were analyzed statistically. The results were that each Dentocult SM degree showed significant variances in incidence rate, as did the dft and caries severity index results in the second examination. The dft and caries severity index of both examinations exhibited a high degree of statistical significance. The same may be said of the Dentocult LB findings for the two years. No noticeable variances in caries incidence rate, dft and caries severity index from the Dentobuff Strip test were observed in both years' study, nor there any statistical significance. The conclusion is that Dentocult SM is the best of the three tests for the diagnosis of the presence of caries and prognosis of its progress, Dentocult LB is second best whereas the Dentobuff Strip shows no detection capability.

Rajtboriraks D, Nakornchaai S, Bunditsing P, Surarit R, Iemjaren P (2004)[54] evaluated the plaque and saliva fluoride level after placement of fluoride releasing Pit and Fissure sealants. Eighteen children aged 6 to 9 years were randomly divided into 2 groups (Group I Helioseal-F and Group II Teethmate-F). Saliva and plaque samples were collected before and after the placement of sealants at 24hours, 9 days, 2 weeks and 4 weeks. Fluoride levels were determined using microdiffusion method. There was no change in salivary fluoride level but the plaque level of Helioseal-F was higher than the baseline level at 24 hours and was not different afterwards. There was no difference between the salivary and plaque fluoride levels of the two groups at different time intervals.

Chi ZB, Gao YX, Pan Y, Zhang B, Feng XP (2004)[14] studied the effect of toothpaste containing IgY against oral Streptococcus mutans in 140

subjects, who were divided into 2 groups randomly (test group and control group). Before the beginning of the test, every subject underwent an elution period in order to lessen the other interference factors and the test began. Streptococcus mutans values were determined with Dentocult SM Strip just before test and at 1, 3, 7, 30 days after toothpaste used and 14 days after stopping toothpaste used. In test group, Streptococcus mutans values decreased at 1 day after toothpaste used. In control group Streptococcus mutans values decreased at 3 days after toothpaste used. Streptococcus mutans values decreased gradually in each groups after toothpaste used. In test group, 2 weeks after stopping toothpaste, Streptococcus mutans values were still suppressed. The application of the toothpaste containing IgY may reduce oral Streptococcus mutans levels significantly.

Karjalainen S, Soderling E, Pienihakkinen K (2004)[35] compared a commercially available strip test with the conventional laboratory assay. Two plaque samples obtained from the mesial surfaces of the upper right and lower left permanent molars of sixty-five 10-year-old children (boys = 38, girls = 27) were cultured and incubated using chair-side site strip tests (Dentocult SM, Orion Diagnostica). Two plaque sampling tools, namely dental floss and micro-brush were compared, and inter-examiner agreement between recordings of three examiners was assessed. Paraffin-stimulated saliva was then collected for laboratory and chair-side assays. The plaque and saliva chair-side tests correlated well with each other (Spearman rho, r = 0.72) and with the laboratory method, showing coefficients of 0.76 and 0.80 for saliva and plaque, respectively. Compared to the laboratory method, the sensitivity (Sn), specificity (Sp), accuracy (A), and kappa (K) values of the salivary and plaque chair-side tests were 0.63, 0.75 (Sn), 0.93, 0.90 (Sp), 0.82, 0.85 (A), and 0.58, 0.66 (K), respectively. Agreement between the two plaque sampling techniques was good (0.91). Inter-examiner agreement of plaque scores ranged between 0.65 and 0.86 when all density categories were analyzed separately; when dichotomized into low and high categories, complete agreement was found. Agreement between the plaque and saliva chair-side tests and the laboratory

salivary assay was good, and in terms of sensitivity, accuracy and kappa values, the site strip plaque test surpassed the salivary chair-side test.

Galaviz LAA, Premoli G, Gonzalez A, Rodriguez RA (2005)[32] evaluated the relationship between the presence of Lactobacillus species and Streptococcus mutans and dental caries in a school children population. The relation of PI- DMFT have a value of significance p=0.001489. In dental caries risk evaluation, the Streptococcus mutans and Lactobacillus species detection in saliva is a good predictor and contributing to the caries development.

MATERIALS AND METHODS

This study was carried out in the Department of Pedodontics and Preventive Dentistry in association with the Department of Microbiology, Meenakshi Ammal Dental College, Chennai.

SAMPLE SELECTION:

All kindergarten children in Arulmigu Meenakshi Amman Matriculation Higher Secondary School, Chennai were screened by examiner (A) using mouth mirror and probe under day light. Forty eight caries free children with full set of primary dentition were selected. The parents of these children were asked to report to the department. The study was explained to the parents in detail. Child's personal details, details of past medical history including any recent antibiotic exposure, past dental history including recent fluoride treatment, frequency of brushing, sweets/snacks intake and consumption of sugared/energy drinks and the brand of toothpaste to know about its fluoride content were obtained through a questionnaire from parents. Thirty subjects (Fig.2) were selected for the study with the following inclusion criteria.

1. Caries free primary dentition
2. No history of antibiotics for the past 3-4 weeks
3. No history of fluoride treatment for the past 2 weeks.

Written consent was obtained from these parents.

Each subject was assigned a specific number by asking them to pick up a lot which was done by examiner (B). The statistician randomized the numbers into the control group and the study group. Every subject had the equal chance of being in both the groups. Group-I (study group) consisted of 20 subjects and Group-II (control group) consisted of 10 subjects. The subjects were blinded about their group to which they belong.

MATERIALS:

The materials used for this study were Dentocult SM kit (Orion Diagnostica, Finland) (Fig.3) and Fluor Protector fluoride varnish (Vivadent, Germany) (Fig.3). From the content of Dentocult SM kit (Fig.4) the following materials were used for this study,

a. 10 strips (flat tipped) for plaque collection
b. 10 selective culture vials
c. 10 labels
d. Bacitracin discs.

The selective culture broth used for the study contained

Tryptose	- 10 g/l
Peptone	- 10 g/l
Glucose	- 1 g/l
Saccharose	- 300 g/l
K_2HPO_4	- 5 g/l
Trypan blue	- 12 mg/l
K- Tellurite(1%)	- 1 ml

PLAQUE SAMPLE COLLECTION:

A fully equipped mobile dental van (Fig.2) of our college was taken to the school for the collection of the plaque sample. The sample was collected 1-2 hours after eating/brushing as it could affect the growth of the bacteria. The kit was removed from the igloo box and brought to room temperature before starting the procedure. The first step was to add the bacitracin discs to the selective culture vials using forceps, 15 minutes before starting the procedure. The bacitracin tube was removed from the foil pack without removing the desiccant. After the desired number of discs were removed, the tube was re-inserted, dispensing end first, into the foil pack. When taking the disc from a previously opened foil pack, the first two or three discs were discarded and the remaining discs were used.

The plaque strips were removed from the pack and the plaque samples were collected using toothpick by examiner (A). They were collected from the following four sites

a. buccal surface of maxillary right molar (Fig.5)

b. labial surface of maxillary incisor (Fig.6)

c. labial surface of mandibular incisor (Fig.7) and

d. lingual surface of mandibular left molar (Fig.8) by different toothpick for the control and study group. These samples were spread thoroughly but gently on the four sites of the rough surface of the strip. The culture vials were shaken to evenly distribute the bacitracin discs. The strips were then placed in the selective culture broth, with the smooth surfaces clipped and attached to the cap. The vials were then labeled as per their lot number and incubated (Fig.9) in an upright position at 37ºC for 48 hours with the cap opened one quarter of a turn. The minimum incubation time was 48 hours for the growth of the organisms.

After the collection of the plaque sample from all the patients, fluoride varnish was applied to the subjects of study group by examiner (B) on the same day (Fig.10). First the tooth surfaces were completely cleaned, dried with air syringe and then isolated with cotton rolls as per the manufacturer's instructions. A high volume evacuator with saliva ejector and cheek retractor were also used. A thin layer of Fluor Protector fluoride varnish was applied on all the tooth surfaces using a suitable brush. The cotton rolls were removed after 1 minute and the patient was asked not to rinse the mouth immediately and not to eat or brush their teeth for 45 minutes. After 24 hours, plaque samples were again collected from the subjects of both the groups in the same van in the school. These were also incubated for the same time as before and the same interpreters evaluated the results again.

INTERPRETATION OF RESULTS:

After incubation the presence of the streptococcus mutans is confirmed by detecting light-blue to dark-blue, raised colonies on the

inoculated surface of the strip. Colonies suspended in the culture broth were excluded from the evaluation. The results were evaluated according to the manufacturers' chart (Fig. 11)

Class 0- <10,000 CFU/ml (CFU- Colony Forming Unit)

Class 1- <100,000 CFU /ml

Class 2- 100,000 -1000 000 CFU /ml

Class 3- > 1000 000 CFU/ ml

The results were interpreted by two independent interpreters (examiner A and C), who were also blinded about the group division. Inspection was done with the growth sideways against a light or with the magnifying glass for raised colonies. The presence of epithelial cells on the strip surface should be differentiated from the mutans colonies. This was done by passing a gloved finger along the strip. The epithelial cells on the strip surface were smooth while the streptococci colonies were rough. Hence only the rough colonies were accounted for the growth of the Streptococcus mutans (Fig.12).

The subjects in the experiment group, the examiner who collected the plaque and the interpreter of the results were blinded about the division of groups. Hence it can be referred as a randomized controlled triple blind study.

ARMAMENTARIUM **(Fig 1)**

Mouth mirror

Explorer

Tweezer

Surgical Mask

Surgical Gloves

Dentocult SM Kit

- Selective Culture vials
- Plaque strips (flat tipped)
- Bacitracin discs
- Labels

Toothpick

Fluor Protector Fluoride Varnish

Applicator Tips and Handle

High-Volume Evacuator

Saliva Ejector

Cheek Retractor

Cotton rolls

Incubator

Questionnaire

QUESTIONNAIRE

NAME:

AGE: **DATE OF BIRTH:**

SEX:

ADDRESS:

PHONE No.:

PMH: History of antibiotics course two to four weeks before **Yes / No**

PDH: History of any fluoride treatment two weeks before **Yes / No**

I. BRUSHING HISTORY:

a. Frequency of brushing: 1 2

b. Type of paste used: COLGATE
CLOSE-UP
PEPSODENT
ANCHOR
OTHERS SPECIFY

c. Is the paste FLUORIDATED 1 (OFFICE USE)
NON-FLUORIDATED 2 (OFFICE USE)

II. DIET HISTORY:

a. Does your child take any of these sweet foods?

CHOCLATES
TOFFIES
CANDIES **YES / NO**
BISCUITS
MILK SWEETS
OTHERS SPECIFY

b. If yes, how many times/ day?

0 ------- 0
1 ------- ≤ 2
2 ------- 3-4
3 ------- > 4

c. Does your child take any of these sweet drinks?

MILK / COFFEE / TEA
FRIUIT JUICES
ENERGY DRINK
SUGARED WATER **YES / NO**
CARBONATED COOL DRINKS
OTHERS SPECIFY

d. If yes how many times / day?

0 ------ 0
1 ------ ≤ 2
2 ------ 3-4
3 ------ > 4

e. Specify any other dietary plan / pattern of your child?

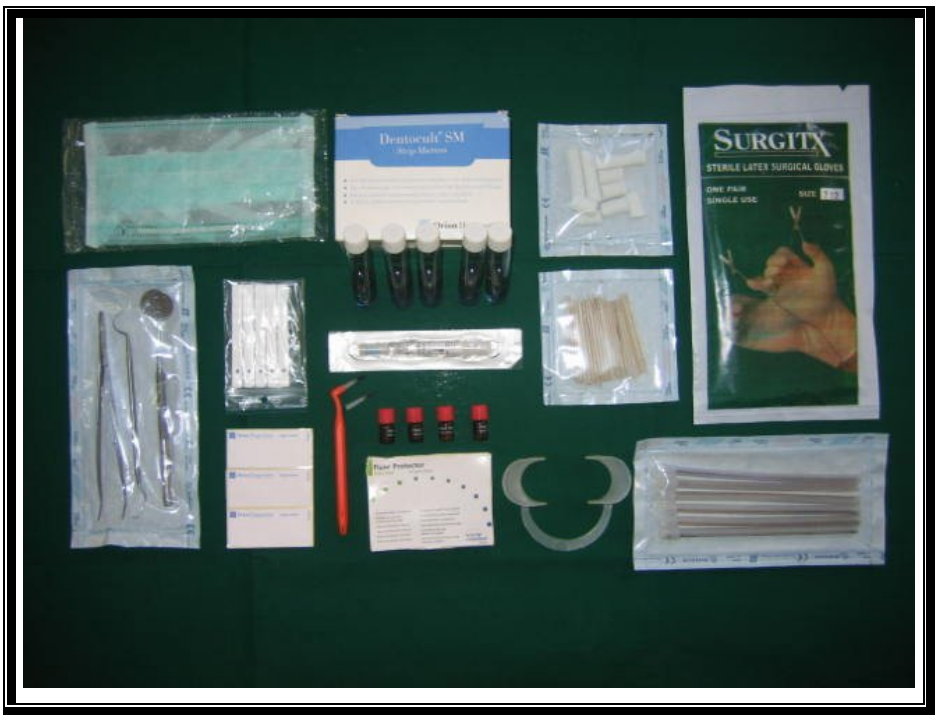

Fig 1: Armamentarium

Fig 2: Part of study sample and Mobile Dental Van

Fig 3. Dentocult- SM Kit and Fluor Protector

Fig 4: Contents of Dentocult-SM Kit

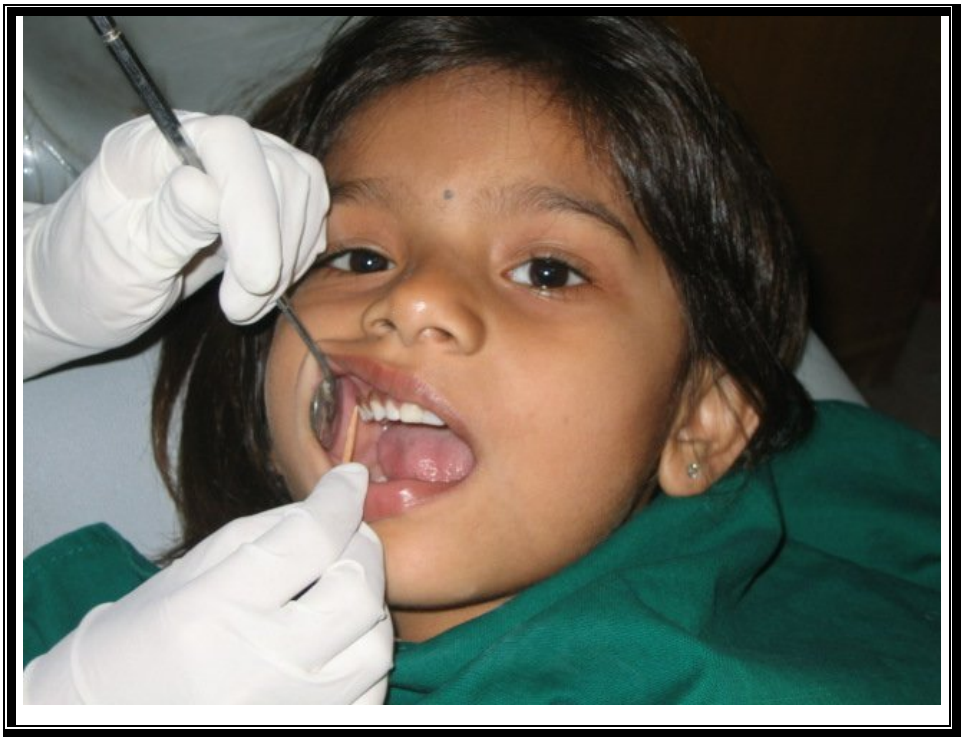

Fig 5: Sample collection- buccal surface of maxillary right molar

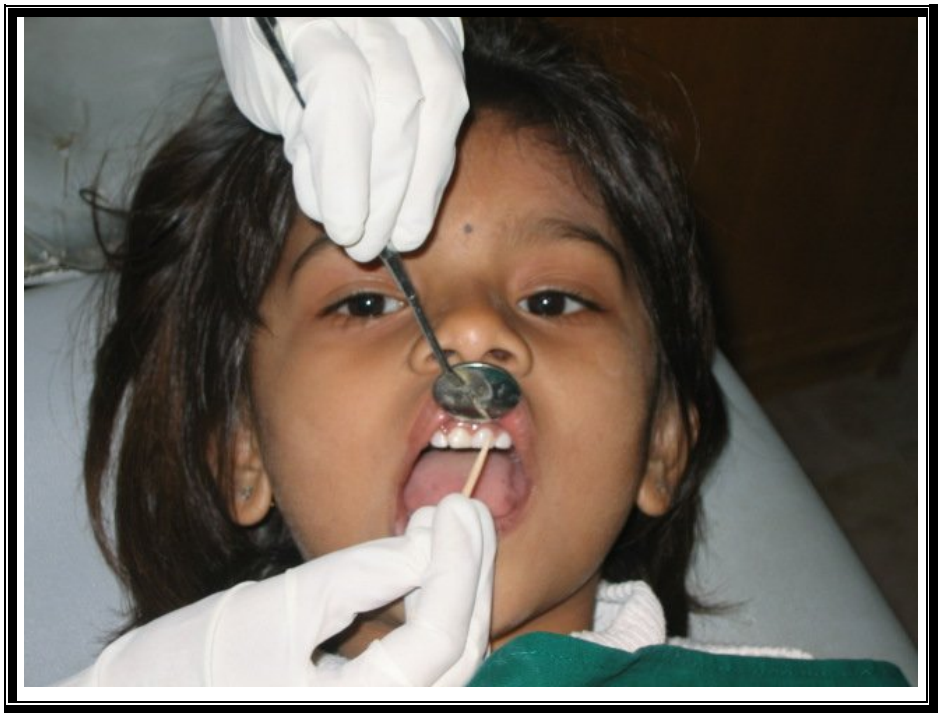

Fig 6: Sample collection- labial surface of maxillary incisor

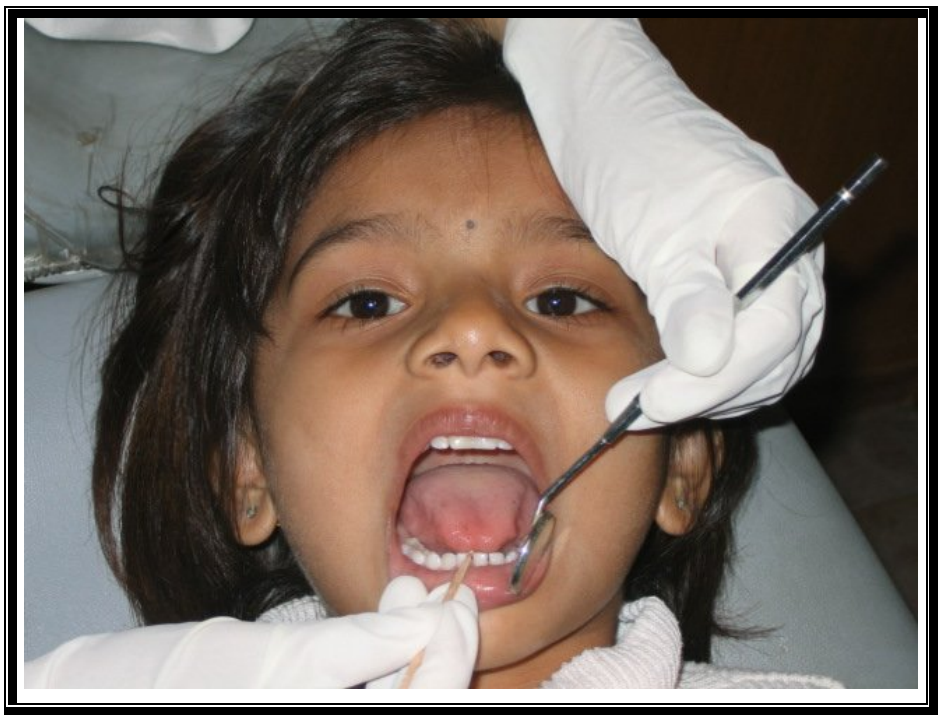

Fig 7: Sample collection- labial surface of mandibular incisor

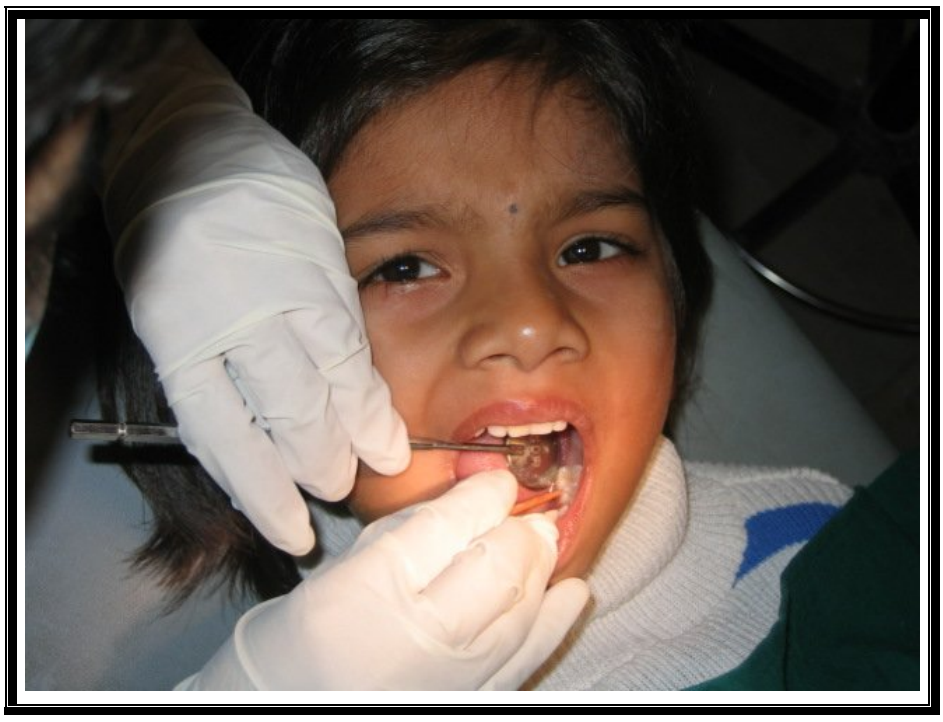

Fig 8: Sample collection-lingual surface of mandibular left molar

Fig 9: Incubator

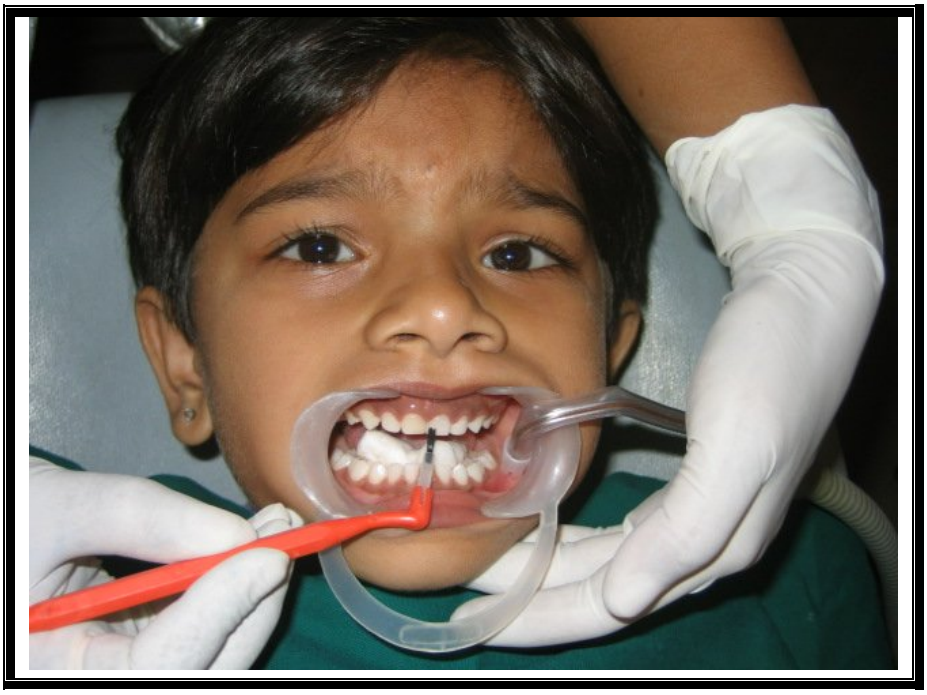

Fig 10: Fluoride varnish application

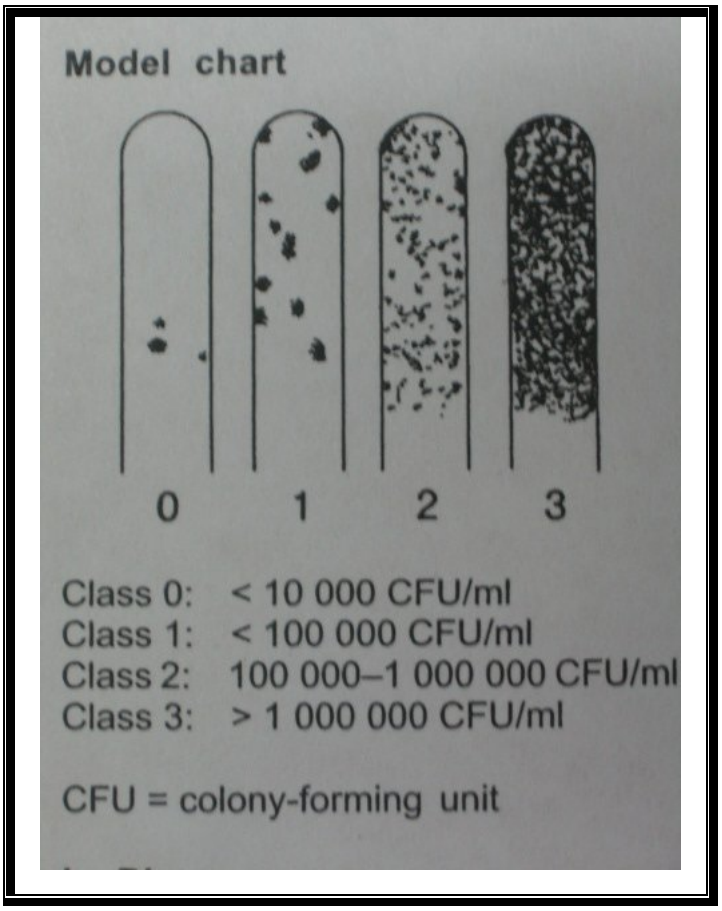

Fig 11: Manufacturer model chart

Fig 12: Different classes (0, 1, 2, 3) of Streptococcus mutans growth

Fig 13: Pre-treatment samples growth after incubation

Fig 14: Post-treatment samples growth after incubation

RESULTS

TABLE I:

Distribution of sample and Mean age in Years:

Sample	No. of Subjects	Mean Age (Yrs) (mean ± sd)
Study Group	20	4.17 ± 0.80
Control Group	10	4.49 ±1.12

Table I shows the distribution of the subjects and their mean age in the study and the control group. There were 20 subjects in the study group and 10 in the control group. The mean age for the study group was 4.17 ± 0.80 (mean ± sd) where as for the control it was 4.49 ±1.12.

TABLE II:

Comparison of Descriptive Variables of Study Group with Pre-treatment Bacterial Score:

S.No.	Variables	Frequency of variables	Value	Bacterial Score Class				Total No. of subjects	p- value < 0.05 (sig)
				0	1	2	3		
1	Frequency of brushing	One / day	1	0	14	5	0	19	0.117* (ns)
		Twice / day	2	0	0	1	0	1	
2	Type of Toothpaste	Fluoridated	1	0	14	6	0	20	–
		Non-Fluoridated	2	0	0	0	0	0	
3	Frequency f Sweets/Snacks	0 / day	0	0	1	0	0	1	0.304* (ns)
		≤ 2 / day	1	0	11	5	0	16	
		3-4/ day	2	0	2	0	0	2	
		> 4 / day	3	0	0	1	0	1	
4	Frequency of Sweet Drinks	0 / day	0	0	0	0	0	0	0.117* (ns)
		≤ 2 / day	1	0	14	5	0	19	
		3-4/ day	2	0	0	1	0	1	
		> 4 / day	3	0	0	0	0	0	

*** Pearson Chi-Square test**

sig- Significant

ns- Not significant

Table II shows the distribution of the various variables with pre-treatment bacterial count in the study group. All the subjects used only fluoridated toothpaste but the frequency of brushing, sweets/snacks intake and sugared drinks varied with the subjects of the study group. There was no statistically significant effect of the above variables on the pre-treatment bacterial count in the study group **(Chi-square p > 0.05)**.

TABLE III:

Comparison of Descriptive Variables of Study Group with Post-treatment Bacterial Score:

S.No.	Variables	Frequency of variables	Value	Bacterial Score Class				Total No. of subjects	p- value < 0.05 (sig)
				0	1	2	3		
1	Frequency of brushing	One / day	1	11	8	0	0	19	0.257* (ns)
		Twice / day	2	0	1	0	0	1	
2	Type of Toothpaste	Fluoridated	1	11	9	0	0	20	–
		Non-Fluoridated	2	0	0	0	0	0	
3	Frequency of Sweets	0 / day	0	1	0	0	0	1	0.279* (ns)
		≤ 2 / day	1	8	8	0	0	16	
		3-4/ day	2	2	0	0	0	2	
		> 4 / day	3	0	1	0	0	1	
4	Frequency of Sweet Drinks	0 / day	0	0	0	0	0	0	0.257* (ns)
		≤ 2 / day	1	11	8	0	0	19	
		3-4/ day	2	0	1	0	0	1	
		> 4 / day	3	0	0	0	0	0	

*** Pearson Chi-Square test**

sig- Significant

ns- Not significant

Table III shows the distribution of the variables with the post-treatment bacterial count. Even though the variables varied with the subjects there was no statistically significant effect of them on the post-treatment bacterial count in the study group using **(Chi-square p > 0.05)**.

TABLE IV:

Distribution of Streptococcus mutans score and average bacterial count for control group using Strip mutans:

Bacterial Score	Pre-treatment		Post-treatment		p- value < 0.05 (sig)*
	No. of Subjects	Average Bacterial Count	No. of Subjects	Average Bacterial Count	
Class 0	0	10^4 to 10^5 **CFU/ml**	1	10^4 to 10^5 **CFU/ml**	**0.455 (ns)**
Class 1	7		7		
Class 2	2		1		
Class 3	1		1		

CFU/ml- Colony forming units/ml

*** Mann-Whitney U test**

sig- Significant

ns- Not significant

Table IV shows the distribution and average pre and post-treatment bacterial count in the control group. There was no statistically significant difference in the distribution of bacterial score in the control group **(Mann-Whitney U test p= 0.455)**. The average pre and post-treatment bacterial count is in the range of 10^4 to 10^5 CFU/ml.

TABLE V:

Distribution of Streptococcus mutans score and average bacterial count for study group using Strip mutans:

Bacterial Score	Pre-treatment		Post-treatment		p- value < 0.05 (sig)*
	No. of Subjects	Average Bacterial Count	No. of Subjects	Average Bacterial Count	
Class 0	0	10^4 to 10^5 **CFU/ml**	11	$>10^4$ **CFU/ml**	**0.000 (sig)**
Class 1	14		9		
Class 2	6		0		
Class 3	0		0		

CFU/ml- Colony forming units/ml

*** Mann-Whitney U test**

sig- Significant

ns- Not significant

Table V shows the distribution and average pre and post-treatment bacterial count in the study group. There was a statistically significant difference in the bacterial count **(Mann-Whitney U test p= 0.000)**. The average pre-treatment bacterial count was about 10^4 to 10^5 CFU/ml where as it is only less than 10^4 CFU/ml after fluoride varnish application.

TABLE VI:

Inter-examiner (A and C) relationship between pre-treatment and post-treatment scores:

Inter-examiner relationship (kappa)	No. of subjects	Pre-treatment	Post-treatment
	30	0.926	0.821

Table VI shows the inter-examiner relationship between the pre-treatment and post-treatment bacterial count in all the subjects. The value was **0.926** and **0.821** using **Cohen's kappa.**

Table VII:

Distribution and Average of Bacterial scores in caries free children:

Bacterial Score	No. of Bacteria (CFU/ml) Colony Forming Units/ml	No. of Subjects	Median	mean ± sd
Class 0	$< 10^4$	0	10^4-10^5	1.33±0.55
Class 1	10^4 - 10^5	21		
Class 2	10^5 - 10^6	8		
Class 3	$> 10^6$	1		

Table VII shows the distribution of the pre-treatment bacterial count in the plaque of caries free primary dentition. The average number of bacteria can be in the range of 10^4-10^5 colony forming units/ml. The mean and the standard deviation for bacterial score were about 1.33±0.55.

DISCUSSION

Fluoride has been found to be the most effective cariostatic agent in the field of dentistry especially in Pediatric dentistry. In the past few decades it has completely changed the approach of treatment from a therapeutic concept to a more preventive approach. The action of fluoride for caries prevention are multiple such as effects on the teeth, bacteria and plaque. In the teeth it alters the physiochemical properties by making it more resistant to acid dissolution due to formation of fluorapatite or fluorhydroxyapatite. It also increases the post-eruptive maturation, enhances remineralisation and inhibits demineralization[30]. In the bacteria it inhibits various enzymes like enolases, phosphatases, proton extruding ATPases and pyrophosphatases[33]. Fluoride also influences the bacterial composition and alters the plaque ecosystem[10]. Plaque fluid, an aqueous phase within the plaque has more concentration of fluoride than any other oral fluids. The sources of fluoride for the plaque fluid are from the calcium fluoride on the enamel beneath the plaque, calcium fluoride in plaque and fluoride in saliva and gingival fluid[25]. Thus plaque can retain and concentrate more fluoride[18,69]. Hence in our study the effect of Fluor Protector fluoride varnish on Streptococcus mutans count in plaque of caries free children was analyzed using Dentocult SM strips.

Subjects (30) with only caries free dentition were chosen for our study to assess the actual effect of fluoride varnish on the Streptococcus mutans count in plaque. Table I shows the distribution of the randomly divided experimental group. It included group-I (study group- 20 subjects) and group-II (control group-10subjects). The mean age of the children in the study group was 4.17 ± 0.80 years where as the mean age for the control group was 4.49 ±1.12 years. These subjects were selected based on the information obtained through the questionnaires filled by the parent. This also had the variable data's regarding the type of toothpaste, frequency of brushing, sweets/snacks intake and sugared/energy drinks. These data were collected in order to know the diet

pattern of subjects and to evaluate their role on Streptococcus mutans count in plaque.

Plaque samples were collected from all the subjects using toothpick and they were incubated. As per the manufacturer model chart the bacterial score were interpreted as Class 0- <10,000 CFU/ml (CFU- Colony Forming Unit / ml), Class 1- 10,000 - 100,000 CFU /ml, Class 2- 100,000 -1000 000 CFU /ml and Class 3- > 1000 000 CFU/ ml. Table II shows the distribution and comparison of the variables in the study group with pre-treatment bacterial count (Fig.13) obtained using Strip Mutans. From the questionnaire it was found that 19 subjects brushed their teeth once except one who brushed twice. Of the 19 subjects the Streptococcus mutans score was class 1in 14 subjects and the remaining were in class 2. The person who brushed twice also belonged to class 2. All the subjects of the study group used only fluoridated toothpaste. There were 14 subjects in class 1 and the remaining in class 2. The distribution of the frequency of sweets/snacks intake also varied between the subjects of the study group. There were 14 subjects in class 1 of which 11 consumed 1-2 times/day, 2 subjects consumed 3-4 times/day and 1 person did not consume at all. There were 6 subjects in class 2 of which 5 consumed 1-2 times/day and 1 consumed more than 4 times per day. In the frequency of sweet drinks, there were 19 subjects who consumed 1-2 times/day of which 14 were in class 1 and 5 in class 2. One subject who consumed 3-4 times sweet drinks/day belonged to class 2. There was no statistical significance between the pre-treatment bacterial score and frequency of brushing, sweets/snacks intake and sugared/energy drinks consumption.

Yoshiara A et al (2001)[81] compared variables like sealants, dfs, DMFS, frequency of sweet drinks, sweets/snacks, and brushing, fluoridated and non-fluoridated paste with the bacterial count while evaluating the effect of fluoride mouthrinse on it. But only frequency of sweets/snacks, dfs and sealants had significant effect on bacterial count. The reason for the significance could be the larger sample size of their study and moreover the bacterial score was also grouped as high (class 2 & 3) and low (class 1 & 2). Hence with the above

dependant variables logistic multiple regressions was carried out to find the actual effect of fluoride mouth rinse on Streptococcus mutans count. But in our study as there was no statistical significant difference found, no such analysis was done. All the subjects of the study group used only fluoridated tooth paste and hence no statistical analysis was done to evaluate its effect on bacterial count. Moreover the subjects of the control group also used fluoridated paste. But according to Peterson et al (1991)[48] there is no difference in the level of mutans streptococci between subjects using or not using fluoridated tooth paste. Yoshiara A and his co-workers too did not find any significant effect of fluoridated tooth paste on the Streptococcus mutans count[81].

Plaque samples were collected from the previously mentioned sites with the aim of assessing the overall effect of fluoride varnish on Streptococcus mutans count. However Vogel and Ekstrand (1992)[80] found that there is a large variation between the plaque fluid fluoride concentrations at various sites of the oral cavity. He collected the plaque sample from the upper and lower molars and incisors after rinsing with 10 ml of 0.2% NaF mouth rinse. Plaque fluid collected in the maxillary incisor region had much higher concentration than any other sites. Considering the above fact and feasibility to collect four sites simultaneously in a plaque strip of Dentocult SM kit, four site samples were taken. According to Shi et al (1998, 2003)[64,65] Dentocult SM is the best test for the diagnosis of the presence of caries and prognosis of it with high statistical significance. Dentocult SM is better than Dentocult LB for caries risk assessment. Moreover, this test is chair side, more patient compliance especially for young age, minimal armamentarium needed, less time consuming and easy sample collection. Davenport ES et al (1992)[17] compared Dentocult SM kit with conventional method and found these dip-slide test provide a simple and suitable method of screening salivary Streptococcus mutans level, which may have a useful role in caries risk assessment. In a similar study by Karjalainen (2004)[35] and his co-workers, the sensitivity, specificity and accuracy of Dentocult SM were better than the conventional

methods. In their study the plaque test surpassed the salivary strip test in terms of sensitivity and accuracy when both were compared.

Fluor Protector fluoride varnish was applied after the collection of pre-treatment plaque sample. Twenty four hours later, post-treatment plaque samples were again collected as before to check and compare the bacterial count. Table III shows the distribution and comparison variables with the post-treatment bacterial count (Fig.14) in the study group. Of the 19 subjects who brushed once, 11 were in class 0 (<10,000 CFU/ml) whereas the remaining belonged to class 1 (10,000 - 100,000 CFU /ml). The person who brushed twice also belonged to class 1. All the subjects of the study group used fluoridated toothpaste. There were 11 subjects in class 0 and the remaining in class 1. In the frequency of sweets/snacks intake, there were 11 subjects in class 0 of which 8 consumed 1-2 times/day, 2 subjects consumed 3-4 times/day and 1 person did not consume at all. There were 9 subjects in class 2 of which 8 consumed 1-2 times/day and one more than 4 times/day. In the frequency of sweet drinks, there were 19 subjects who consumed 1-2 times/day of which 11 belong to class 0 while others in class 1. One subject who consumed 3-4 times sweet drinks/day belonged to class 1. There was no statistically significant difference between the post-treatment bacterial score and frequency of brushing, sweets/snacks intake and sugared/energy drinks consumption. According to Ekenbach SB et al (2000)[23] who studied the colonization of cariogenic bacteria in plaque of exposed root surfaces after application of various varnish found no statistically significant difference between baseline and over time (1 week, 1 and 6 months) samples with Fluor Protector. Hence a twenty four hour plaque sample was collected after which fluoride varnish was even applied to the control group. This was done so that they are not deprived of the preventive effect of fluoride varnish. This study was triple blinded as explained in materials and methods to eliminate bias and to get more authenticated results.

Table IV and V shows the distribution of the pre and post-treatment bacterial score in the control group and study group. Of the 10 subjects in

control group the pre-treatment score was class 1(10,000 - 100,000 CFU /ml) in 7 subjects, 2 subjects were in class 2 (100,000 -1000 000 CFU /ml) and one subject was in class 3 (> 1000 000 CFU/ ml). In the post-treatment count 7 subjects were in class1 and one subject each in other classes. There was no statistically significant difference between the pre and post-treatment bacterial scores in the control group (Mann Whitney test p= 0.455 ns). The average pre and post-treatment bacterial count of the control group is in the range of 10^4-10^5 CFU/ml. Of the 20 subjects in study group, the pre-treatment bacterial count was class 1 (10,000 - 100,000 CFU /ml) in 14 subjects and the remaining were in class 2 (100,000 -1000 000 CFU /ml). After fluoride varnish (Fluor Protector) application there were only 9 subjects in class 1 while the others were in class 0 (<10,000 CFU/ml). This was statistically significant (Mann Whitney test p=0.000 sig). The average pre-treatment bacterial count of the study group was in the range of 10^4-10^5 CFU/ml where as the post-treatment count is only less than 10^4 CFU/ml. According to Killian et al (1979)[37,38], naturally occurring fluoride does not significantly influence the bacterial composition of plaque but higher levels of fluoride could eliminate susceptible micro-organism and modify the plaque ecosystem. Sköld-Larsson and his co-workers compared fluoride concentration of three varnishes in plaque and found some of the members of Fluor Protector group showed increased level of fluoride in plaque even after 30 days[66]. Munshi AK et al (2001)[46] in an in-vitro study evaluated the demineralizing inhibitory and antibacterial effect of Fluor Protector and found that it has the highest demineralising inhibitory effect and least antibacterial effect when compared to Bifluoride-12 and Fluoritop SR. Ekenbach SB and co-workers (2001)[24] found that pre-treated hydroxyapatite crystals with Fluor Protector produced statistically significant reduction in lactic acid formation in Streptococcus mutans. Hence the reduction of the bacterial count in this study could be due to high concentration of fluoride from Fluor Protector which might have entered the bacterial cell and resulted in the inhibition of various cellular processes. This fluoride could have inhibited enolase which indirectly affects the formation of ATP, which is central to cell

maintenance and growth. It could have also inhibited H^+/ATPase in it which compromises the maintenance of cellular pH and makes the internal environment more acidic and unsuitable for other enzymes to act. Fluoride also inhibits exogenous glycerol uptake into lipoteichoic acid which is believed to play an important role in membrane stability and in the colonization of Streptococcus mutans on hydroxyapatitie. It could have also reduced the peptidoglycan macromolecule in the cell membrane and there by causing partial lysis of cell membrane.

However, Zickert I and Emilson CG (1982)[84] found that Duraphat did not have any significant effect on plaque and salivary levels of Streptococcus mutans on 4th, 10th and 21st days after treatment in preschool children. Here they have not evaluated the 24 hour action of Duraphat on plaque sample and moreover they could have used a different medium and method to estimate the count. Eventhough Fluor Protector has low concentration of fluoride and caries inhibiting activity when compared to Duraphat, the fluoride deposited in teeth was more in Fluor Protector than Duraphat[61,62]. This fluoride which could have leached out from the teeth could have been taken up by the plaque to inhibit the growth of bacteria. Brown et al (1983)[12] showed that Streptococci were eliminated in 10 out of 30 patients, whose plaque had 115 ppm of fluoride. Berg et al (1990)[7] and Forss et al (1991)[31] found lower levels of Streptococcus mutans in plaque adjacent to fluoride releasing glass ionomers. According to Songpaisan et al (1994)[67], children using 0.5% hydrofluoride solution showed low level of Streptococcus mutans. In an in-vitro study by Munshi AK et al (2001)[46] where they compared the antibacterial effect of Bifluorid 12, Fluoritop-SR and Fluor Protector in Blood agar and MSBA (Mitis Salivarius Bacitracin Agar), it was found that Fluor Protector had the least inhibitory effect. This was due to the low fluoride content of Fluor Protector when compared to other fluoride varnishes. Yoshiara A et al (2001)[81] concluded that the long term use of fluoride mouth rinses had significant antibacterial action on the Streptococcus mutans.

The inter-examiner reliability for the pre and post-treatment bacterial count was found to be 0.926 and 0.821 using Cohen's kappa (Table VI). Table VII shows the distribution of the pre-treatment bacterial scores for all the samples. Of the thirty subjects 21 were in class 1, 8 in class 2 and one belonged to class 3. The median for the bacterial score was class 1 and the mean and standard deviation was about 1.33±0.55. Thus an average number of colony forming units in caries free children with primary dentition could be about 10^4 to 10^6. Levels accepted as risk for caries in adults and older children per ml of stimulated saliva was about 10^5 to 10^6 [39,45,57,79].

SUMMARY AND CONCLUSION

The aim of the study was to evaluate the effect (24 hour) of Fluor Protector fluoride varnish on the Streptococcus mutans count in plaque of caries free primary dentition using Dentocult SM test and to estimate the Streptococcus mutans count in them. Hence thirty caries free children with full set of primary dentition were selected and divided into study group (20 subjects) and control group (10 subjects). The conclusions derived from the results of this study are

- Fluor Protector fluoride varnish have a statistically significant reduction in the Streptococcus mutans count in plaque after 24 hours.
- The average Streptococcus mutans count in primary dentition of caries free children is in the range of 10^4 to 10^5 colony forming units/ml.
- The average Streptococcus mutans count in primary dentition of caries free children after Fluor Protector fluoride varnish application is below 10^4 colony forming units/ml.
- Frequency of brushing, sweets/snacks intake, sugared/energy drinks consumption and the use of fluoridated toothpaste do not have any statistically significant effect on the Streptococcus mutans count of plaque.

As this was the first study, further studies can reveal the long term effect of Fluor Protector fluoride varnish on the Streptococcus mutans count in plaque of carious and caries free dentition.

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