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Comparative evaluation of chlorhexidine varnish and fluoride varnish on plaque *Streptococcus mutans* count – an *in vivo* study

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Sajjan PG, Nagesh L, Sajjanar M , Reddy SKK, Venktesh UG. Comparative evaluation of chlorhexidine varnish and fluoride varnish on plaque *Streptococcus mutans* count – an *in vivo* study.

Abstract: *Aim:* The aim of the study was to assess and compare the effect of chlorhexidine varnish and fluoride varnish application on *Streptococcus mutans* counts in plaque of occlusal pits and fissures of permanent mandibular first molars. *Materials and methods:* The study was an *in vivo* comparative study, conducted among 50 schoolchildren aged 7–8 years under a field setting. The 50 subjects were randomly allocated into two groups. Baseline plaque samples were collected from all the subjects followed by the application of two varnishes, Cervitec and Duraphat. The varnish was applied to pit and fissures of occlusal surface of mandibular first molar. The varnish application was carried out on the first day, fifth day and tenth day after baseline plaque sampling. Subsequent plaque samples were collected at the end of 1 month and at the end of 3 months after the varnish application. *Results:* The Cervitec varnish has shown a statistically significant reduction at the end of 1 month and at the end of 3 months ($P < 0.05$). Duraphat varnish did not show a statistically significant difference in reducing the plaque *S. mutans* count at the end of 1 month and third month ($P > 0.05$). *Conclusion:* Cervitec varnish was found to be effective in reducing *S. mutans* count for a 3-month period, when compared to Duraphat varnish.

Key words: caries; chlorhexidine varnish; dental caries; fluoride; gel; micro-organism; *S. mutans*

Introduction

The goal of dentistry is to help the individuals in achieving maximum oral health throughout their life. Major challenge for achieving this goal is the widespread nature of dental diseases, mainly dental caries and periodontal disease. Successful attempts were made to combat high incidence of dental caries in developed countries, but the increased incidence of dental caries is still a great burden in the developing countries (1). Although dental caries is not a life-threatening disease, it still has great biological, physical, social and economic implications. Effective methods to control caries hence receive a great attention.

Dental caries is a multifactorial disease. Various host, agent and environmental factors play an important role in the development of dental caries. In recent days, various strategies for controlling dental caries focus on disrupting the interaction between all the risk factors that are thought to play an important role in the dental caries. Some of these measures

include dietary modification, adequate oral hygiene practices, use of fluorides, pit and fissure sealants and use of antimicrobial agents.

All such measures directly or indirectly target the oral microflora. Some of the direct measures for controlling dental caries include the use of antimicrobial agents to suppress the growth of microorganisms responsible for dental caries. A group of microorganisms are thought to play an important role in the dental caries. Among all the species, *Streptococcus mutans* (*S. mutans*) has found to be a predominant organism responsible for dental caries. *S. mutans* are predominantly found in retentive areas such as carious lesions and pit and fissures of teeth. Among all the surfaces of the tooth, pit and fissure areas harbour increased proportion, about 70%, of *S. mutans*. Longitudinal studies have found a significant association between increased proportion of *S. mutans* in saliva and dental plaque and incidence of dental caries (2, 3).

Epidemiological evidence suggests that incidence of dental caries is high among children (4) It has also been reported in the literature that pits and fissures of occlusal surfaces, especially of mandibular permanent first molar in children, are favourable sites for the development of dental caries (5). Some of the reasons attributed to this are morphology of pits and fissures, which makes them inaccessible to tooth brush bristles, and lack of manual dexterity among young children to effectively use tooth brush (6, 7). Newly erupted teeth undergo post-eruptive maturation and are highly vulnerable to the development of dental caries before they undergo complete maturation (8). So tooth protection becomes crucial during this stage.

Research in the field of caries prevention has been focused on targeting the plaque microflora, which can work independent of patient compliance. Various antimicrobial agents have been tested and tried to reduce or eliminate the plaque flora completely. One such successful agent is chlorhexidine, a bisbiguanide salt, which acts by selective suppression of *S. mutans* leaving other bacteria unaffected. Chlorhexidine in the form of mouthwash and gel has found to be effective in reducing the level of microorganisms, but faster recovery of microorganisms to original level was a frequent observation (9, 10). Moreover, the use of these two preparations is associated with side effects such as staining and altered taste sensation (11, 12).

However, with the use of chlorhexidine in the form of varnish, the level of microorganisms in saliva and dental plaque was suppressed for extended period of time and it was found to be associated with fewer side effects when compared to mouthwash and gel (13–17). The degree of reduction was based upon the concentration of chlorhexidine varnish used and its frequency of application. Many studies have reported that chlorhexidine varnish has significantly reduced the *S. mutans* levels in saliva and approximal plaque(18–21), but studies on the effect of chlorhexidine varnish on the level of *S. mutans* in pits and fissures of occlusal plaque are limited (22–24). Results of these studies indicate a wide variation in the period of suppression of *S. mutans* depending upon the concentration of chlorhexidine used in the varnish and also upon the frequency of applications. The lapse in knowledge base pertaining to exact concentration and frequency of application served as an area for the exploration in the current study.

The combination of chlorhexidine and fluoride varnish has shown an increased period of suppression of *S. mutans* in saliva than chlorhexidine varnish alone, providing some evidence of synergistic effect and indicating the possible role of fluoride varnish in reducing *S. mutans* counts (18). Some other studies have tested the antimicrobial efficacy of sodium fluoride varnish, but the results showed that there was no significant effect (25–28). Several *in vitro* and *in vivo* studies have shown that the concentration of fluoride in the plaque after topical fluoride application can interfere with metabolic activity of *S. mutans* and thereby reduces the growth of *S. mutans*, but the evidence is not clear (29). The effect of sodium fluoride mouth rinse, sodium fluoride gel and APF gel application on *S. mutans* in saliva and dental plaque has been tested, but the results were not encouraging (30–32).

Davangere city, which is selected for the present study, is known to have high prevalence of dental caries among children and increased proportion of subjects with pit and fissure caries (33). As the studies have shown that level of *S. mutans* in saliva and dental plaque is directly proportional to incidence of pit and fissure caries (34), the strategies for effective control of caries in these areas include effective suppression of *S. mutans* for a sustained period. Thus, in this regard, an effort is made in this study to assess and compare the effect of chlorhexidine varnish and fluoride varnish application on *S. mutans* level in dental plaque of occlusal pits and fissures.

Methodology

The present study is an experimental study conducted to evaluate the effect of chlorhexidine varnish and fluoride varnish application on *S. mutans* counts in dental plaque of occlusal pits and fissures of mandibular first permanent molars among selected schoolchildren aged 7–8 years in Davangere city as measured by the difference in the level of 'colony-forming units' of *S. mutans* at different time intervals such as at the baseline, at the end of 1 month and at the end of 3 months.

The study proposal was submitted for approval and clearance from the ethical review board of Bapuji Dental College and Hospital, Davangere. All the required and relevant information about the number and location of government lower primary schools in Davangere city was obtained from the office of DDPI, Davangere. Voluntary written informed consent was obtained from the parents of selected 7- to 8-year-old schoolchildren.

Sample size determination based upon pilot study results

The 'α' (probability of committing type I error, significance level) and 'β' (probability of committing type II error) were fixed well in advance by the investigator at ≤ 0.05 and $\leq 20\%$, respectively. The power of the study being $1-\beta$ was maintained at 86%. Based on these values, the minimum sample

size recommended for each group was 20. In the present study, a total of 25 subjects in each group were considered. It accounted for a total sample size of 50 subjects.

The kappa coefficient value for interexaminer variability with respect to *S. mutans* count was 0.87. Davangere city was arbitrarily divided into four zones (north-east, north-west, south-east and south-west). From each of the zones, two schools were selected randomly by employing lottery dip method. Hence, eight schools were selected of four zones. A total of two schools were randomly selected from eight schools using lottery dip method. Block randomization method was used, and each school was considered as a block. In each school, a total of 25 children were randomly selected from a group of children who fulfilled the eligibility criteria, and thus, there were two blocks. Cervitec varnish and Duraphat varnish application constituted two interventions for two groups. The two groups are randomly allocated under two arms of experimental trial, Group 1 – Cervitec varnish – and Group 2 – Duraphat varnish.

Inclusion criteria were:

1 Subjects should belong to 7- to 8-year age group.
2 Subjects should have fully erupted mandibular first permanent molars with no evidence of caries.

Exclusion criteria were:

1 Subjects who were on antimicrobial therapy for any time in the last 3 months.
2 Subjects who are suffering from any systemic illness.
3 Subjects who were on any artificial fluoride supplementation or on any antimicrobial mouth rinse for the last 6 months.
4 Subjects who were physically and mentally handicapped.

Plaque sampling and varnish application methods

Prior to plaque sampling, at baseline, at the end of 1 month and at the end of the 3 months, the subjects were informed to refrain from oral hygiene procedures for 24 h. Baseline plaque samples were obtained from the selected teeth of the selected subjects just before the application of allocated varnish. Adherent saliva on the test teeth was removed by a gentle blow of air. Disclosing solution (alpha plac; Dental Products of India, Mumbai, India) was applied on the occlusal surface of both the mandibular first molars using a cotton pellet to facilitate easy identification of plaque. A sterile arch explorer (Shepard's hook end, No 23) was used to collect the plaque from occlusal pits and fissures of every mandibular first permanent molar (35). All the available plaques from the occlusal pits and fissures of both the mandibular first molars on either side were collected separately and, with the help of sterile wooden tooth pick, transferred into two small separate test tubes containing 100 μl of normal saline. All the above-mentioned procedures were performed by the investigator under field setting with proper aseptic measures.

The plaque samples were processed within 1 h. Samples were homogenized manually by stirring using a stirrer. Inoculating loop with a diameter of 0.05 μl was used to collect the diluted plaque from test tube. Plaque samples were spread over MSB culture media (Mitis Salivarius supplemented with bacitracin and 20% sucrose), a selective medium for *S. mutans*, and incubated for 48 h at 37°C in an incubator (36). After 48 h of incubation period, *S. mutans* appeared on the culture plate as small, rough, raised and adherent colonies. Those colonies that were atypical were further confirmed by mannitol and sorbitol test. Colonies so identified were counted using an electronic colony counter (Deep Vision Company, Chennai, India). All the microbiological procedures were performed by microbiology technician, who was blinded to culture plates of different groups and the group from which the plaque was collected. The colony-forming units per ml of diluted plaque were measured based on the values obtained per 100 μl of diluted plaque.

After baseline plaque samples were obtained from each of the subject in both the groups, respective varnish application procedure was carried out for each of the subject. Before the application of varnish, the selected teeth of both groups were thoroughly cleaned by passing a jet of water. They were then isolated with cotton rolls and dried with gentle blow of air using chip blower. Group I was treated with Cervitec varnish (containing 1% w/w chlorhexidine diacetate and 1% w/w thymol as active ingredients, Vivacare, Schaan Liechtenstein), and Group II was treated with Duraphat (containing 5% of sodium fluoride; Colgate Palmolive Company, New York, NY, USA). Approximately 0.1 ml of the designated varnish was applied to the occlusal pits and fissures of mandibular first molars of both the groups. A small brush supplied by the manufacturer was used for Cervitec group. For Duraphat varnish groups, small cotton pellets were used for the application of varnish. Varnish was allowed to dry, and after 25 s, cotton rolls were removed (18). Subjects in both groups were instructed not to drink or eat anything for 3 h and not to brush on those teeth for 24 h.

The varnish application was repeated at fifth day and tenth day for both the groups. The follow-up plaque samples from both the varnish groups were taken at the end of 1 month and at the end of 3 months in the same manner as described before and assessed for *S. mutans* counts using above-mentioned microbiological procedures. During the study period, the subjects were asked to report any adverse effects noticed by them immediately to the school authorities and the investigator. However, none of the subjects reported any adverse effects or side effects.

Statistical analysis

Pairwise comparisons of plaque *S. mutans* counts of two groups at baseline, 1 month and 3 months were made by unpaired *t*-test. Comparison of plaque *S. mutans* counts at baseline, at the end of 1 month and at the end of 3 months for each group was made by paired *t*-test. Comparison of plaque *S. mutans* counts within the groups at baseline and at the follow-up period of 1 month and 3 months was made by one-way ANOVA followed by intergroup comparison by *post hoc* test.

Results

The mean colony counts of *S. mutans* for both Cervitec and Duraphat varnish groups at different follow-ups are shown in Fig. 1. At baseline, the levels were 8.476 × 10^5 ml^{-1} of diluted plaque for Cervitec varnish group and 8.356 × 10^5 ml^{-1} of diluted plaque for Duraphat varnish group. At the end of 1 month, the levels were 2.316 × 10^5 ml^{-1} of diluted plaque for Cervitec varnish group and 8.280 × 10^5 ml^{-1} of diluted plaque for Duraphat varnish group. At the end of third month, *S. mutans* counts were 4.432 × 10^5 ml^{-1} of diluted plaque for Cervitec varnish group and 8.552 × 10^5 ml^{-1} of diluted plaque for Duraphat varnish group. Table 1 shows that there was no significant difference ($P > 0.05$) between both groups at baseline. Table 2 shows that at the end of 1 month, there was a significant difference ($P < 0.05$) between Cervitec and Duraphat varnish groups. Table 3 shows that at the end of third month, there was a significant difference between both the groups ($P = 0.000$). The results of one-way ANOVA on *S. mutans* count in the plaque are summarized in Table 4. There was a significant difference at different follow-ups for Cervitec varnish group ($P = 0.000$ $P < 0.05$). *Post hoc* test (Tukey–Kramer) showed that there was significant difference from baseline to 1 month, from baseline to 3 months and from 1 month to 3 months. Table 5 shows the results of one-way ANOVA for Duraphat varnish group. There was no significant difference in Duraphat varnish group at different follow-ups (0.920, $P > 0.05$).

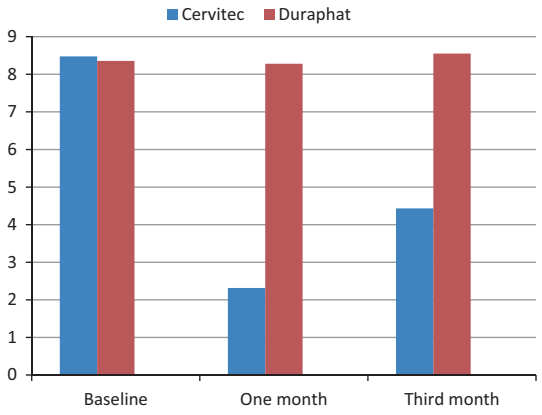

Fig. 1. Comparison of mean plaque *Streptococcus mutans* count for both Cervitec and Duraphat varnish groups at different follow ups at baseline, 1 month and third month.

Table 1. **Comparison of mean plaque *Streptococcus mutans* count between Cervitec varnish and Duraphat varnish group at the baseline**

Groups	Mean	SD	SE	*t* value	*P* value	Significance
Group 1	8.476	1.909	0.381	0.215	0.831	NS
Group 2	8.356	2.031	0.406			

NS, Non-significant.
S. mutans count is generally presented as the 'mean unit ×10^5 cfu ml^{-1}' of diluted plaque.
$P > 0.05$.

Table 2. **Comparison of mean plaque *Streptococcus mutans* count between Cervitec varnish and Duraphat varnish group at the end of 1 month**

Groups	Mean	SD	SE	*t* value	*P* value	Significance
Group 1	2.316	0.968	0.193	11.923	0.000	HS
Group 2	8.280	2.305	0.461			

HS, highly significant.
$P < 0.05$.

Table 3. **Comparison of mean plaque *Streptococcus mutans* count between Cervitec varnish and Duraphat varnish group at the end of third month**

Groups	Mean	SD	SE	*t* value	*P* value	Significance
Group 1	4.432	1.465	0.293	6.376	0.000	HS
Group 2	8.552	2.879	0.575			

HS, highly significant.
$P < 0.05$.

Discussion

This study was conducted under field setting including a total of 50 subjects belonging to 7- to 8-year age group representing two schools (25 subjects in each group) that were randomly selected from a list of all the schools in Davangere city. The study employed block randomization method. Each school was considered as a block. As the two test materials were different in their colour, consistency and taste, there was no other alternative to blind the participants with respect to two different test materials used in the study except by applying block randomization.

The study aimed to test the effect of two varnishes in real-life situation without exercising too many controls on subject characteristics such as dietary practices, socioeconomic status and oral hygiene practices. The study being community trial has made an attempt to test the selected two varnishes under field conditions unlike clinical trial. The present study results can be attributed to whole varnish and not to active ingredients alone, as the study design does not incorporate suitable negative control.

The present study aimed to assess and compare the effect of chlorhexidine varnish and fluoride varnish on *S. mutans* counts in dental plaque of occlusal pits and fissures of mandibular first permanent molars, and there is no scope for attributing obtained results exclusively to active ingredients in the varnishes.

The caries susceptibility is high for newly erupted permanent first molar (15, 37–39). Dirk *et al.* reported that 83.3% of permanent first molars are carious by 8 years of age (39). Considering the high prevalence of caries in the first 2 years after eruption due to post-eruptive maturation, 7- to 8-year age group is preferred in the present study.

Cervitec varnish was found to be effective in reducing plaque *S. mutans* count at the end of 1 month and at the end of 3 months, but the mean plaque *S. mutans* score at the end of 3 months shows a tendency to shift towards baseline values.

Table 4. **Mean plaque *Streptococcus mutans* count measured as colony-forming units at baseline, at the end of 1 month and at the end of 3-month interval for Cervitec varnish group**

Study groups	Mean	*F* value	*P* Value	Significant	*Post hoc*
Baseline One month Three months	8.476 2.316 4.432	109.13	0.00	HS	Significant difference between baseline and 1 month, baseline and third months, 1 month and third months

HS, highly significant.
$P < 0.05$.

Table 5. **Mean plaque *Streptococcus mutans* count measured as colony-forming units at baseline, at the end of 1 month and at the end of 3-month interval for Duraphat varnish group**

Study groups	Mean	*F* value	*P* Value	Significant
Baseline One month Three months	8.356 8.280 8.552	0.083	0.920	NS

NS, non-significant.
$P > 0.05$.

Similar results were obtained in some other studies (15, 16, 22, 23). In the studies of Joharji *et al.* (22), Bratthall *et al.* (23) and Araujo *et al.* (15), the use of Cervitec varnish application on pits and fissures on monthly intervals showed the suppression of *S. mutans* counts for a period of 3 months. In the study of Joharji *et al.* using Cervitec varnish, suppression up to 9 months was observed, but last application was carried out at the end of 6 months, indicating the overall period of suppression of 3 months. In the present study, three applications within 2-week interval were carried out and the suppression for 3-month period was observed. Based on these observations, it can be speculated that intensive application of three times within 2-week interval has similar efficacy compared to monthly applications.

In comparison, studies on the use of Cervitec varnish on plaque *S. mutans* of interproximal areas showed that the intense applications of chlorhexidine varnish have better effect compared to monthly applications (40, 41). In the study of Ekenback *et al.* (26), the *S. mutans* count was reduced in the plaque samples of root carious lesions for a period of 1 month.

The present study results are in conformity with the abovementioned study results; although some of the studies report the efficacy of Cervitec varnish in reducing the plaque *S. mutans* of other tooth surfaces, the overall effect on *S. mutans* and the period of suppression were found to be similar.

The suppression of *S. mutans* in the plaque is dose dependent (42, 43). Few of the studies have observed longer period of suppression with high concentration of chlorhexidine varnish, but the safety dose and tolerance dose need further evaluation. So, in the present study, a lower concentration of chlorhexidine was used. The present study is a short-term study of 3 months. A long-term study would also have been designed using few more periodic estimations to evaluate the precise period of suppression. This is one of the limitations of the study.

In the present study, Duraphat varnish has not shown a significant antimicrobial effect on *S. mutans* level. Similar results were observed in the study of Zickert and Emilson (27), Ekenback *et al.* (26) and Shaecken *et al.* (25). In the study of Zickert and Emilson (1982), plaque samples were collected from smooth surface and *S. mutans* counts were evaluated at fourth, tenth and twenty-first day after Duraphat varnish application. The study is first of its kind in assessing *S. mutans* count in the plaque of pit and fissures. In the study of Ekenback (26), *S. mutans* level in plaque collected from root caries was determined. In the study of Shaeken *et al.* (25), the plaque samples were obtained from smooth surfaces and evaluated for six consecutive weeks.

At the end of 1 month, the mean plaque *S. mutans* counts between the two groups showed a significant variation. Cervitec was found to be superior to Duraphat. Similar results were observed in the studies of Ekenback *et al.* (26), Shaecken and Haan (24), S Twetman *et al.* (21), Sandham *et al.* (44). At the end of 3-month period, a statistically significant difference was observed between the two groups with reference to mean plaque *S. mutans* counts. Cervitec group was found to be maximum benefited when compared to Duraphat group. The pairwise comparison clearly revealed the superior effect of Cervitec in reducing plaque *S. mutans* counts, when compared to Duraphat. Similar results were observed in the study of Peterson *et al.* (20).

In the present study, evaluation of effect of Duraphat varnish on plaque *S. mutans* count has shown that there is a small, nonsignificant reduction in *S. mutans* count at the end of 1 month. Fluoride may have an initial bactericidal effect on *S. mutans*, but sustainability of effect might be lacking. As the study has not estimated the immediate effect on *S. mutans* count, the effect of Duraphat varnish on *S. mutans* cannot be underestimated. The precise effect of fluoride varnish on plaque *S. mutans* count needs to be evaluated through further studies. The lack of efficacy can also be attributed to minimal amount of plaque on the pit and fissure areas due to thorough cleaning before the application of Duraphat varnish and insufficient amount of fluoride in the plaque to show a significant effect.

Few of the studies have employed splitmouth technique (15, 22, 23), which is undoubtedly the most powerful design to eliminate all the individual variations in plaque microflora. The present study is an active controlled trial and Duraphat varnish was used as a control. If the splitmouth technique is employed, there would be a probable contamination of respec-

tive tooth with the other varnish through saliva. Then, the question would arise whether results obtained are due to the synergistic effect of two varnishes or the effect of single varnish. To avoid the probable biasing of results, the technique is not employed in the present study.

The study limits its findings and conclusion only to the effect of selected varnishes on the *S. mutans* count in the occlusal fissures of lower first molars. The caries preventive effect of these varnishes may be a function of variation in *S. mutans* load. As dental caries is a disease of complex aetiology, which is dictated and influenced not just by *S. mutans* counts but also by an array of host factors and environmental factors, further studies are required to clarify the role of these varnishes in the prevention of dental caries.

Conclusions

The following conclusions can be drawn from the present study.

The application of Cervitec varnish to occlusal pits and fissures of permanent mandibular first molars at the application frequency of three times within 2-week interval can successfully reduce plaque *S. mutans* counts for a period of 3 months and Cervitec varnish has provided maximum benefit against *S. mutans* when compared to Duraphat varnish. Probably if the study was further extended, few more periodic estimations, if conducted, would reveal precisely the exact period for which the suppression of *S. mutans* might continue. If one can determine the optimum period for which Cervitec may suppress *S. mutans*, it will help in planning a preventive regimen including periodic Cervitec applications, which may contribute to the promotion of oral health and prevention of dental caries.

References

1 Cohen KL, Gift CH. *Textbook of Disease Prevention and Oral Health Promotion*. Copenhagen: Munksgaard; 1995.

2 Zickert I, Emilson CG, Krasse B. Correlation of level and duration of *Streptococcus mutans* infection with incidence of dental caries. *Infect Immun* 1983; **39**: 982–985.

3 Zickert I, Emilson C, Krasse B. The effect of caries preventive measures in children highly infected with bacterium S*treptococcus mutans*. *Arch Oral Biol* 1982; **27**: 861–868.

4 Ronald L. Ettinge, Epidemiology of dental caries. Cariology, *Dent Clin North Am* 1999; **43**: 679–694.

5 Powell LV. Caries prediction: a review of literature. *Community Dent Oral Epidemiol* 1998; **26**: 361–367.

6 Raadal M, Laegried KV, Hveem H, Wangen K. Evaluation of a routine for prevention and treatment of fissure caries in permanent first molars. *Community Dent Oral Epidemiol* 1990; **25**: 5–16.

7 Unkel JH, Fenton SJ, Hobbs GJ, Frere CL. Toothbrushing ability is related to age in children. *J Dent Child* 1995; **61**: 346–348.

8 Driessens FCM, Heijligers HJM, Borggreven JMPM, Woltgens JHM. Posteruptive maturation of tooth enamel studied with the electron microprobe. *Caries Res* 1985; **19**: 390–395.

9 Emilson CG. Potential efficacy of chlorhexidine against mutans streptococci and human dental caries. *J Dent Res* 1994; **73**: 682–689.

10 Schaeken MJ, De Jong MH, Franken HC, Van der Hoeven JS. Effects of highly concentrated stannous fluoride and chlorhexidine regimes on human dental plaque flora. *J Dent Res* 1986; **65**: 57–61.

11 Fardal O, Turnbull RS. A review of the literature on use of chlorhexidine in dentistry. *J Am Dent Assoc* 1986; **18**: 863–869.

12 Johnson BT. Uses of chlorhexidine in dentistry. *Gen Dent* 1995; **43**: 126–132.

13 Matthijs S, Adriaens PA. Chlorhexidine varnishes: a review. *Jol Clin Periodontol* 2002; **29**: 1–8.

14 Balanyk TE, Sandham H. Development of sustained-release antimicrobial dental varnishes effective against *Streptococcus mutans* in vitro. *J Dent Res* 1985; **64**: 1356–1360.

15 Araujo AMPG, Naspitz GMCC, Chelotti A, Cai S. Effect of Cervitec on mutans streptococci in plaque and on caries formation on occlusal fissures of erupting permanent molars. *Caries Res* 2002; **36**: 373–376.

16 Riebero LGM. The effect of different formulations of chlorhexidine in reducing levels of mutans streptococci in oral cavity: a systematic review of literature. *J Dent* 2007; **35**: 359–370.

17 Schaeken MIM, Schouten MI, Van Der Kieboom CWA, Van Der Hoeven JS. Influence of contact time and concentration of chlorhexidine varnish on mutans streptococci in interproximal dental plaque. *Caries Res* 1991; **25**: 292–295.

18 Twetman S, Petersson LG. Efficacy of a chlorhexidine and a chlorhexidine-fluoride varnish mixture to decrease interdental levels of mutans streptococci. *Caries Res* 1997; **31**: 361–365.

19 Twetman S, Petersson LG. Interdental caries incidence and progression in relation to mutans streptococci suppression after chlorhexidine-thymol varnish treatments in schoolchildren. *Acta Odontol Scand* 1999; **57**: 144–148.

20 Petersson LG, Maki Y, Twetman S, Edwardsson S. Mutans streptococci in saliva and interdental spaces after topical applications of an antibacterial varnish in schoolchildren. *Oral Microbiol Immunol* 1991; **6**: 284–287.

21 Twetman S, Hallgren A, Petersson LG. Effect of an antibacterial varnish on mutans streptococci in plaque from enamel adjacent to orthodontic appliances. *Caries Res* 1995; **29**: 188–191.

22 Joharji RM, Adenubi JO. Prevention of pit and fissure caries using an antimicrobial varnish: 9 month clinical evaluation. *J Dent* 2001; **29**: 247–254.

23 Bratthall D, Serinirach R, Rapisuwon S *et al*. A study into the prevention of fissure caries using an antimicrobial varnish. *Int Dent J* 1995; **45**: 245–254.

24 Schaeken MJM, De Haan P. Effects of sustained- release chlorhexidine acetate on the human dental plaque flora. *J Dent Res* 1989; **68**: 1786–1789.

25 Schaeken MI, Van Der Hoeven JS, Hendriks JCM. Effects of varnishes containing chlorhexidine on the human dental plaque flora. *J Dent Res* 1989; **68**: 40–44.

26 Ekenback S, Linder LE, Lonnies H. Effect of four dental varnishes on the colonization of cariogenic bacteria on exposed sound root surfaces. *Caries Res* 2000; **34**: 70–74.

27 Zickert I, Emilson CG. Effect of fluoride containing varnish on S*treptococcus mutans* level in plaque and saliva. *Scand J Dent Res* 1982; **90**: 423–428.

28 Muerman JH. Ultra structure, growth, and adherence of S*treptococcus mutans* after treatment with chlorhexidine and fluoride. *Caries Res* 1998; **22**: 283–287.

29 Hamilton IR. Effect of fluoride on enzymatic regulation of bacterial carbohydrate metabolism. *caries Res* 1977; **11**: 262–291.

30 Mayhew RR, Brown LR. Comparative effect of SnF_2, NaF and $Sncl_2$ on growth of S*treptococcus mutans*. *J Dent Res* 1981; **60**: 1809–1814.
31 Brown LR, White JO, Horton IM, Dreizen S, Strekfus JL. Effect of continuous fluoride gel use on plaque fluoride retention and microbial activity. *J Dent Res* 1983; **62**: 746–751.
32 Mayers M, Handleman S. Effect of daily application of fluoride in custom fitted mouth piece on plaque flora associated with dental decay. *J Dent Res* 1971; **50**: 597–599.
33 Sogi G, Bhaskar D. J Dental caries and oral hygiene status of 13–14 year old school children of Davangere. *J Indian Soc Pedod Prev Dent* 2001; **19**: 113–117.
34 Marsh PD. Microbial aspects of dental plaque and dental caries. *Dent Clin North Am* 1999; **43**: 599–613.
35 Sandham HJ, Brown J, Phillips HI, Chan KH. A preliminary report of long-term elimination of detectable mutans streptococci in man. *J Dent Res* 1988; **67**: 9–14.
36 Gold OG, Jordan HV, Van Houte J. A Selective medium for S*treptococcus mutans*. *Arch Oral Biol* 1973; **18**: 1357–1364.
37 Warnakulasuriya S. Caries susceptibility of first permanent molars and treatment needs in Sri Lankan children, aged 13–16 years, in 1986. *Community Dent Health* 1991; **8**: 167–172.
38 King NM, Shaw L, Murray JJ. Caries susceptibility of permanent first and second molars in children aged 5–15 years. *Community Dent Oral Epidemiol* 1980; **8**: 151–158.
39 Dirks OB. Post eruptive changes in dental enamel. *J Dent Res* 1966; **45**: 503–511.
40 Schaeken MJM. Effect of single and repeated application of chlorhexidine varnish on mutans streptococci in plaque from fissures of premolar and molar teeth. *Caries Res* 1993; **27**: 303–306.
41 Twetman S, Petersson LG. Effect of different chlorhexidine varnish regimens on mutans streptococci levels in interdental plaque and saliva. *Caries Res* 1997; **31**: 189–193.
42 Attin R, Tuna A, Attin T, Brunner E, Noack M. Efficacy of differently concentrated chlorhexidine varnishes in decreasing mutans streptococci and lactobacilli counts. *Arch Oral Biol* 2003; **48**: 503–509.
43 Zang Q, Mulder J, Truin GJ, Van Palenstein Helderman WH. Effect of 40% chlorhexidine varnish on mutans streptococci counts in pits and fissures of permanent first molars. *J Dent* 2008; **35**: 589–592.
44 Sandham HJ, Brown J, Chan KH, Phillips HI, Burgess RC, Stokl AJ. Clinical trial in adults of an antimicrobial varnish for reducing mutans streptococci. *J Dent Res* 1991; **70**: 1401–1408.