Preventing the transfer of Streptococcus mutans from primary molars to permanent first molars using chlorhexidine

Sumer M. Alaki, BDS, MS Walter J. Loesche, DMD, PhD Robert J. Feigal, DDS, PhD
Marcio A. da Fonesca, DDS, MS Kathleen Welch, MA, MPH

Dr. Alaki was a graduate student, Department of Pediatric Dentistry; Dr. Loesche is professor, Schools of Dentistry and Medicine; Dr. Feigal is professor, director, and associate dean for graduate programs and facilities, and Dr. da Fonseca is clinical assistant professor, Department of Orthodontics and Pediatric Dentistry, School of Dentistry; Ms. Welch is computer system consultant, Center for Statistical Consultation and Research, University of Michigan, Ann Arbor, Mich. Correspond with Dr. Alaki at sum@umich.edu

Abstract

Purpose: The purpose of this study was to determine if the application of 1% chlorhexidine-containing wax on primary molars during the period of eruption of the first permanent molars could prevent the transfer of certain oral flora, namely Streptococcus mutans, to the permanent molars.

Methods: Fourteen children with a mean age of 6.5 years (7 males and 7 females) were assigned into two groups: a chlorhexidine group (n=9) in which 1% chlorhexidine-containing wax was painted on primary molars on one side of the mouth; and a placebo wax group (n=5) in which a similar wax, but without chlorhexidine, was painted on primary molars on the other side of the mouth. Baseline saliva samples and pooled plaque samples from the primary molars on both sides of the dentition were obtained from the two treatment groups. Following treatment, plaque samples from the occlusal fissures of the first permanent molars on both sides of the dentition were obtained. The levels of S.mutans and other members of the oral flora on the treated sides (chlorhexidine or placebo) were compared with those on the untreated sides.

Results: The results showed that the proportions of S.mutans to S.sanguinis were significantly lower in the chlorhexidine-treated sides compared to the untreated (P=0.04) and in the chlorhexidine-treated patients compared to placebo (P=0.029).

Conclusions: Since lower mutans to sanguinis ratios have been associated with lower caries experience, treating primary molars with 1% chlorhexidine wax during eruption of permanent first molars may be a simple means for shifting the fissure flora of the permanent molars towards a more favorable balance.*(Pediatr Dent 24:103-108, 2002)*

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Dental fissures present a unique bacterial ecology in the mouth. Their method of colonization as well as composition differs from that of smooth and approximal tooth surfaces.[1] Their response to antimicrobial intervention aimed at controlling its bacterial content and caries susceptibility has shown wide variability depending on the type of fissure as well as the method of antimicrobial treatment.[2]

Studies of natural and artificial fissures showed that these surfaces become populated with microorganisms within 24 hours following exposure to the oral environment.[3] Streptococci seem to be the dominant flora in the fissures,[4] and their colonization is believed to be related to a concentration threshold in saliva.[5] *S. mutans* appears to prefer dental fissures as their natural habitat where its percentage has been shown to increase with time compared to the other fissure flora.[6] In addition to the microbial flora, food particles,[7] parts of the enamel organ[8] and areas of mineralization have also been found in the dental fissures.[9]

S.mutans has been implicated by multiple studies as the main bacterial cause in fissure caries. Loesche et al demonstrated an association between plaque levels of *S.mutans* in

occlusal fissures and caries status of the same fissures in children.[10] Bratthall et al showed that a lower percentage of fissures developed caries (2%) where the *Mutans streptococci* score was low compared to fissures heavily populated with *Mutans streptococci* (33%).[11]

For bacteria to successfully colonize the human oral cavity, they must have the ability to adhere to oral surfaces, a property which has been repeatedly shown to be highly specific.[12] In addition to specific surface adhesion, bacteria have the property of coaggregation which allows cell-to-cell recognition of genetically distinct cell types.[13,14] While specific known mechanisms exist for colonization of smooth tooth surfaces,[15] there is less information concerning the colonization of fissures. Fissure colonization may takes place by passive capturing so that organisms which get to the fissures first will be able to lodge themselves into the depth of the fissures.[1] Saliva[16] and neighboring or opposite teeth[17] are believed to be the main sources of infection with *S.mutans* to dental fissures. This is important to know because it provides the basis for this study which targeted the primary molars as a source of infection to erupting permanent first molars.

Multiple approaches have been attempted in an effort to control bacterial composition of dental fissures as a measure of controlling caries development at these sites. Among the most widely used was topical application of chlorhexidine onto the fissures. Schaeken et al[18] applied a varnish containing 40% chlorhexidine diacetate onto selected fissures and found that there was a significant reduction in numbers of *S.mutans* which lasted up to one month after varnish application. Similar results were shown by Le and Schaeken[2] using 40% chlorhexidine varnish with one and two applications onto the fissures of molars and premolars. Suppression of *S.mutans* in this study lasted for up to four months. Despite the good results obtained by these studies and others, *S.mutans* tended to reappear on treated surfaces and sometimes rebound to pretreatment levels.[19]

Not all microorganisms are equally sensitive to chlorhexidine. The amount and duration of suppression by various chlorhexidine applications show considerable variation between different microorganisms. Among the highly susceptible ones are *S.mutans, Staphylococci and Streptococcus salivarius,* whereas some of the less-susceptible ones include *Streptococcus sanguinis*, *Veillonella* and *Proteus.*[20] Different concentrations and the number of applications of chlorhexidine can suppress *S.mutans* to varying degrees. Varnishes, for example, have been shown to suppress *S.mutans* for up to two years,[21] whereas the effect of mouthwashes can last for nearly two weeks.[22]

The following summarizes the concept behind this research. It is presumably possible to create an environment with a low *S.mutans* count around permanent first molars during the colonization of their occlusal fissures. This could be established by controlling one of the main sources of infection to the erupting permanent molars, the adjacent primary molars. Applying 1% chlorhexidine-containing wax to primary molars during eruption of the permanent first molars would diminish the number of *S.mutans* residing on the primary molars to levels below the colonization threshold. Therefore, when the permanent first molars erupt, it will be difficult for *S.mutans* to colonize their occlusal fissures. Other less cariogenic members of the oral flora would presumably fill the fissure space and act as a defense line against *S.mutans.*

Therefore, the aim of the study was to investigate if the application of 1% chlorhexidine-containing wax on primary molars during the eruption of permanent first molars could prevent the transfer of certain oral flora, namely *S.mutans*, to the occlusal fissures of permanent molars.

Methods

Fourteen children participated in this study and were recruited from the Pediatric Dentistry Clinic at the School of Dentistry, University of Michigan. The included children fulfilled the following criteria:

1. Age ranged from 5-7 years with two erupting permanent first molars in the same dental arch (only cusp tips of these molars needed to be clinically visible). Adjacent and opposing primary molars needed to be present.
2. No medical problems necessitating antibiotic prophylaxis.
3. No behavioral problems necessitating sedation.
4. Legal guardian understood English or had an interpreter to explain the nature of the study.

All procedures included within this research were reviewed and approved by the Institutional Review Board at the University of Michigan. The procedures, possible discomfort and risks as well as benefits were explained to the parents of participating children and their consent was obtained prior to the investigation.

The treatment material used in the study, Orastar™ (Castle Beach Company, California) consisted of two waxes, a treatment and a placebo. Both were composed of microcrystalline wax, a transfer agent which aids in binding the wax to the teeth, and a mineral Oil Viscosity Modifier. The treatment wax had an additional 1% chlorhexidine. The two waxes were not exactly similar in their physical characteristics (mild color and texture differences). According to the manufacturer, both waxes could be applied to wet and dry tooth surfaces by a swab, brush or gloved finger and would remain adherent for nearly eight days under normal functions.

In vivo studies showed that Orastar™ (without chlorhexidine) managed to reduce *S.mutans* colonization on rats' teeth treated once daily, 5 days/week.[23] The presence of chlorhexidine in the treatment wax is believed to have an additional benefit of delivering chlorhexidine and maintaining it on primary molars for a prolonged period which would enhance the longevity of the antimicrobial action. After receiving the waxes from the manufacturer, they were randomized by a co-investigator using a randomization table and given to the primary investigator who carried out all the clinical procedures.

At the initial visit, a stimulated saliva sample was collected from all children by requesting them to chew on a piece of sterile utility wax. The defs was recorded for all participants. The primary investigator chose two permanent first molars in the same dental arch (maxillary or a mandibular) to focus on. The selection was based on the two permanent first molars where only cusp tips were clinically visible. These molars were to be evaluated after wax treatment of all adjacent and opposing primary molars. During the next visit, which was scheduled soon after the first one, two separate pooled plaque samples were taken from primary molars, one from each side of the mouth. Each sample included both maxillary and mandibular molars and was done using a separate set of sterile 27-gauge needles and dental floss. The samples were then stored in two tubes containing reduced transport fluid (RTF). The primary molars were cleansed with prophy brushes and dampened pumice (one set for each side of the mouth).

The treatment waxes were randomly assigned to the patients using a random number generator maintained by an individual not involved in either the clinical or laboratory procedures. The clinical examiner was blinded to the treatment assignments, but as the chlorhexidine wax was slightly more opaque than the placebo wax, this blindness could not be assured. As the laboratory and statistical personnel were blinded to the treatment groups, the design could at least be single-blinded in nature.

The primary investigator applied the waxes to the primary molars of each child following the randomization order. Each child either received a chlorhexidine or placebo wax treatment on the primary molars on one side of the mouth while the other side remained untreated. This split-mouth design allowed one side of the mouth (untreated) to act as control for the other. The investigator used a gloved finger to apply the waxes on dried molars. Each child was given a new toothbrush and parents were instructed to use it and discard the old ones. Other postoperative instructions given were to avoid cleaning the primary molars for five days and to avoid using fluoridated mouthwashes until the end of the study.

Children returned after 1-4 months for evaluation. The investigator evaluated the eruption of the two specified permanent first molars. When the occlusal fissures were fully visible, they were both separately sampled using 27-gauge needles and the two samples were then stored in two separate plastic tubes containing RTF.

In the lab, saliva and plaque samples were dispersed by sonication for 30 seconds and 1 ml of this dilution was added to 9 ml of RTF. This dilution, in turn, was mixed by vortexing for 30 seconds and 1 ml was added to 9 ml of RTF. This 10-fold serial dilution was repeated one more time so as to have 1:10, 1:100 and 1:1000 dilutions. A spiral platter was used to plate all dilutions on flagyl (*S.mutans, S.sanguinis, S.salivarius* and total counts), ETSA (*F.nucleatum, capnocytophaga, A.odontolytic*, total anaerobes), TSCB (*S.mutans* and *S.sobrinus*), Sabaraud agar for yeast and LBS for lactobacilli.[10,24,25]

Table 1. Comparison of Mean Bacterial Counts in Primary Molars with that in the Occlusal Fissures of Permanent First Molars in the Chlorhexidine Group (Focusing on the Treated Sides of the Mouth)

Bacterial parameters	Primary molars (baseline) (A)	Permanent molars (post-treatment) (B)	Mean difference (B-A)	Paired t test *P*-value
S.mutans	4.42 ±1.49	2.96 ±1.11	-1.46	0.07
S.sanguinis	4.73 ±1.83	3.34 ±1.32	-1.39	0.008*
Total facultative	7.13 ±0.56	5.83 ±0.46	-1.3	0.005*
Total anaerobes	7.34 ±0.51	5.92 ±0.44	-1.42	0.002*

*Asterisks reflect statistical significance

Culture plates were incubated in an anaerobic chamber (85% N_2, 10% H_2 and 5% CO_2) at 35^{0}C for 5-7 days. The Sabaruad media used to culture yeast was incubated in an aerobic chamber for 5-7 days. The number of colony-forming units (CFU)/ml saliva was counted for each culture plate via a disectomy microscope using the third or fourth sector in the culture plate. The investigator carrying out the culturing and analyses of the microbiological data was unaware of the treatment groups of subjects.

Although multiple bacterial parameters were measured, statistical analysis was limited to parameters which showed abundance in participating children.

Results

The study spanned a period of one year and two months and included a total of 14 children equally divided between the two genders. The mean age of the participants was 6.6 years in the chlorhexidine group and 6.4 years in the placebo group. Participants' assignments using the random number generator resulted in the allocation of 9 children to the chlorhexidine group and 5 to the placebo group. The mean defs was 20 in the CHX group and 13 in the placebo group. Seven children in the chlorhexidine group had detectable *S.mutans* in their saliva at the beginning of the study, whereas all children in the placebo group had detectable *S.mutans*. None of these differences showed statistical significance.

The statistical analysis was carried out using SPSS statistical software (version 8 for Windows). Four bacterial parameters were included in the statistical analysis: *S.mutans, S.Sanguinis*, total facultative organisms and total anaerobes. These counts were converted into log base 10 values to stabilize the variance and produce a more symmetric distribution. Paired-samples t tests were used to compare bacterial parameters between the two sides of the mouth, treated and untreated, while independent-samples t tests were used to compare parameters between chlorhexidine and placebo groups. No statistically significant differences were found between bacterial counts in the occlusal fissures of the permanent first molars on the treated sides of the mouth (in

Table 2. Comparison of Mean Bacterial Counts in Primary Molars with that in the Occlusal Fissures of Permanent First Molars in the Placebo Group (Focusing on the Treated Sides of the Mouth)

Bacterial parameters	Primary molars (baseline) (A)	Permanent molars (post-treatment) (B)	Mean difference (B-A)	Paired t test *P*-value
S.mutans	4.03 ±2.02	4.46 ±1.19	0.42	0.75
S.sanguinis	3.44 ±1.46	3.8 ±1.64	0.35	0.77
Total facultative	6.65 ±0.73	5.62 ±1.96	-1.03	0.2
Total anaerobes	6.81 ±0.81	5.91 ±1.67	-0.9	0.2

chlorhexidine and placebo groups) compared to the untreated.

The same bacterial parameters in primary molars (baseline levels) were compared to those in the occlusal fissures of permanent first molars (post treatment). The aim of this comparison was to verify if wax treatment had any effect on regulating the transfer of flora from the primary to the permanent molars. Tables 1-4 show a comparison of bacterial counts between the occlusal fissures of permanent first molars and primary molars (numbers presented as log base 10 values). Tables 1 and 2 focus on the treated sides of the mouth while Tables 3 and 4 focus on the untreated sides.

It can be seen from Table 1 that in the sides of the mouth treated with chlorhexidine wax, all bacterial parameters were significantly lower (marked by asterisks) in the permanent first molars with the exception of *S.mutans*, which showed no significant difference. Sides treated with placebo wax (Table 2) demonstrated variations in the differences of bacterial counts but did not show any statistical significance. Table 3 shows that children who received chlorhexidine treatment also had significantly lower bacterial counts in permanent first molars in the untreated sides. Children who received the placebo wax (Table 4) had significantly lower counts for total facultative and total anaerobes.

The ratio of *S.mutans* to *S.sanguinis* (mut/sang) in primary and permanent first molars was calculated by dividing the absolute counts of *S.mutans* by that of *S.sanguinis*. The log base 10 values of the *S.mutans*/*S.sanguinis* ratios on permanent first molars was calculated and used to compare the treated with the control sides (paired t tests) in each study group (Table 5) then the comparison was done between the two study groups using independent t tests (Table 6). Table 5 shows that the mut/sang ratio was higher in the control sides of the chlorhexidine and placebo wax patients compared to the treatment sides. Paired-samples t test showed that the mean difference between the sides was only significant for the chlorhexidine group (P=0.04). Table 6 shows that the *S.mutans*/*S.sanguinis* ratio was lower in the chlorhexidine group on both sides. The difference, however, was statistically significant only for the treatment sides (P= 0.029).

Discussion

S.mutans has long been implicated in the etiology of dental caries. Loesche et al demonstrated the association between high levels of S.mutans in occlusal fissures of children and the presence of caries.[24] The present study adopted a new method for preventing the colonization of occlusal fissures of permanent first molars with *S.mutans.* Data from Caufield et al[26] suggest that there may be a finite window of infectivity associated with eruption of the permanent first molars, a hypothesis supported by other research.[27,28]

While 12 bacterial parameters were measured in the study, only four were used for statistical analysis which included *S.mutans, S.sanguinis*, total facultative organisms and total anaerobes. The reason for excluding other organisms was that only negligible amounts were found in most children, which made statistical analysis inapplicable. Some of these parameters were non-existent in some of the children.

Comparing bacterial counts on treatment sides between primary and permanent first molars showed significant

Table 3. Comparison of Mean Bacterial Counts in Primary Molars with that in the Occlusal Fissures of Permanent First Molars in the Chlorhexidine Group (Focusing on the Untreated Sides of the Mouth)

Bacterial parameters	Primary molars (baseline) (A)	Permanent molars (post-treatment) (B)	Mean difference (B-A)	Paired t test *P*-value
S.mutans	4.5 ±1.17	3.48 ±1.36	-1.02	0.04*
S.sanguinis	4.53 ±1.36	3 ±1.19	-1.53	0.003*
Total facultative	6.96 ±0.54	5.29 ±1.31	-1.67	0.004*
Total anaerobes	7.09 ±0.57	5.31 ±1.48	-1.78	0.004*

*Asterisks reflect statistical significance

Table 4. Comparison of Mean Bacterial Counts in Primary Molars with that in the Occlusal Fissures of Permanent First Molars in the Placebo Group (Focusing on the Untreated Sides of the Mouth)

Bacterial parameters	Primary molars (baseline) (A)	Permanent molars (post-treatment) (B)	Mean difference (B-A)	Paired t test *P*-value
S.mutans	4.15 ±1.41	4.56 ±0.99	0.4	0.7
S.sanguinis	3.62 ±1.54	3.29 ±1.36	-0.33	0.8
Total facultative	6.75 ±0.39	5.47 ±0.52	-1.28	0.006*
Total anaerobes	6.93 ±0.56	5.54 ±0.54	-1.39	0.007*

*Asterisks reflect statistical significance

Table 5. Comparison of *S.mutans*/*S.sanguinis* Ratios BetweenTreatment and Control Sides (Numbers Presented as Log Base 10 Values of the Actual Ratios)

Group	Mut/sang ratio treatment sides (A)	Mut/sang ratio control sides (B)	Mean difference (A-B)	Paired t test *P*-value
Chlorhexidine	-0.38 ±0.69	0.6 ±1.4	-0.98	0.04*
Placebo	0.66 ±0.79	1.27 ±1.77	-0.61	0.52

*Asterisks reflect statistical significance

Table 6. Comparing *S.mutans*/*S.sanguinis* Ratios Between Study Groups (Numbers Presented as Log Base 10 Values of the Actual Ratios)

Group	Mut/sang ratio in chlorhexidine group (A)	Mut/sang ratio placebo group (B)	Difference (A-B)	Independent t test *P*-value
Treatment	-0.38 ±0.69	0.66 ±0.79	-1.05	0.029*
Control	0.49 ±1.4	1.27 ±1.8	-0.79	0.37

*Asterisks reflect statistical significance

reduction (with exception to *S.mutans*) on the permanent molars in the chlorhexidine group. It is plausible that chlorhexidine may have suppressed the bacterial counts on primary molars to levels extremely low, making them unable to invade the fissures of the permanent molars. The finding that bacterial counts were significantly lower in the permanent first molars on the control sides in the chlorhexidine group was interesting. This change may be explained by the antibacterial effect acquired by the saliva due to its contact with the chlorhexidine wax and would be in agreement with data obtained from Sandham et al.[29] In their study, a chlorhexidine varnish was applied to the teeth of 51 adult volunteers once weekly for four weeks. This treatment resulted in significant reduction in the levels of salivary *S.mutans* (99.9% reduction) when compared to a placebo varnish treatment or prophylaxis.

The ratio of *S.mutans* to *S.sanguinis* was instrumental to the study. Our knowledge of these species indicates that *S.mutans* correlates positively with incidence of dental caries while *S.sanguinis* correlates negatively.[30] Data from Sandham et al shows that patients treated with chlorhexidine varnish experienced an increase in their salivary *S.sanguinis* count while *S.mutans* count decreased.[29] Therefore, we used this ratio as our main microbiological indicator. A negative ratio would indicate that the treatment was effective while a positive ratio would reflect an ineffective treatment. It can be seen from Tables 5 and 6 that the chlorhexidine-treated sides were the only areas which showed a negative *S.mutans*/*S.sanguinis* ratio and were significantly different from the other treated or control sides.

These results suggest that the chlorhexidine wax was effective in lowering the mut/sang ratio in the treated sides of the mouths, shifting it towards a presumably less cariogenic balance. Unlike *S.mutans, S.sanguinis* is believed to have superior ability to attach to tooth surfaces. Previous data indicate that it was difficult for *S.mutans* to colonize and sustain itself when introduced into the mouths of adult volunteers who have a stable plaque biota.[31] Therefore, it is the authors' belief that once *S.mutans* is eliminated from the environment during the initial colonization of the permanent first molars' fissures, it would be difficult for it to reestablish itself in the future.

The value of this research could be summarized in the following:

1. Chlorhexidine was used in a new form which is easy to apply, inexpensive and believed to have an extended duration of action due to its prolonged adherence to the teeth.
2. Chlorhexidine wax treatment could be reinstituted again with eruption of the second permanent molars hoping to achieve similar microbiological effects.
3. Previous research used chlorhexidine to treat already colonized teeth. Despite successful results, there was a tendency for bacterial counts to reestablish pretreatment levels. In this study, the reduced levels of *S.mutans* in the occlusal fissures are believed to remain suppressed by the competing flora of the fissures.

The study however, was limited in its inability to recruit more subjects during the allocated time. Since the effects of treatment were primarily based on the microbiological aspects, an area for future research would be to evaluate the effects of treatment on caries status of the occlusal fissures of permanent first molars. Also, longer follow-up periods of participants may be recommended for future research to determine stability of bacterial counts following treatment.

Conclusions

Treating primary molars with 1% containing chlorhexidine wax during eruption of permanent first molars may be a simple means for shifting the fissure flora of the permanent molars towards a more favorable balance.

References

1. Loesche W. Dental Caries: A Treatable Infection. Automated Diagnostic Documentation, Inc., 1993, pp 93.
2. Le YL, 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* 27:303-306, 1993.
3. Thott EK, Folke LE, Sveen OB. A microbiologic study of human fissure plaque. *Scand J Dent Res* 82:428-436, 1974.
4. Meires JC, Schachtele CF. Fissure removal and needle scraping for evaluation of the bacteria in occlusal fissures of human teeth. *Dent Res* 63:1051-1055, 1984a.
5. Igarashi K, Lee IK, Schachtele CF. Effect of dental plaque age and bacterial composition on the pH of

artificial fissures in human volunteers. *Caries Res* 24:52-58, 1990.
6. Theilade E, Fejerskov O, Karring T, Theilade J. A microbiological study of old plaque in occlusal fissures of human teeth. *Caries Res* 12:313-319, 1978.
7. Karring T, Ostergaard E, Theilade J, Loe H. Histochemical study of the formation of dental plaque in artificial fissures. *Scand J Dent Res* 82:471-483, 1974.
8. Newman HN. The pre-eruptive portion of the human enamel integument. *J Dent* 3:110-120, 1975.
9. Theilade J, Fejerskov O, Horsted M. A transmission electron microscopic study of 7-day old bacterial plaque in human tooth fissures. *Arch Oral Biol* 21:587-598, 1976.
10. Loesche WJ, Rowan J, Straffon LH, Loos P. Association of Streptococcus mutans with human dental decay. *Infect and Immun* 11(6):1252-1260, 1975.
11. Bratthall D, Serinirach R, Rapisuwon S, Kuratana M, Luangjarmekorn V, Luksila K, Chaipanich P. A study into the prevention of fissure caries using an antimicrobial varnish. *Int Dent J* 45:245-254, 1995.
12. Whittaker CJ, Klier CM, Kolenbrander PE. Mechanisms of adhesion by oral bacteria. *Annu Rev Microbiol* 50:513-552, 1996.
13. Kolenbrander PE, Ganeshkumar N, Cassels FJ, Hughes CV. Coaggregation: specific adherence among human oral plaque bacteria. *Faseb J* 7(5):406-413, 1993.
14. Kolenbrander PE. Coaggregation among oral bacteria. *Methods in Enzymology* 253:385-397, 1995.
15. Kolenbrander PE. Oral microbial communities: biofilms, interactions and genetic systems. *Annu Rev Microbiol* 54:413-437, 2000.
16. Svanberg M, Loesche WJ. The salivary concentration of Streptococcus mutans and Streptococcus sanguis and their colonization of artificial fissures in man. *Arch Oral Biol* 22:441-447, 1977.
17. Svanberg M, Loesche WJ. Implantation of Streptococcus mutans on tooth surfaces in man. *Arch Oral Biol* 23:551-556, 1978.
18. Schaeken MJ, Beckers HJ, Hoeven JS. Effect of chlorhexidine varnish on Actinomyces naeslundii genospecies in plaque from dental fissures. *Caries Res* 30:40-44, 1996.
19. Kozai K, Wang DS, Sandham HJ, Phillips HI. Changes in strains of mutans streptococci induced by treatment with chlorhexidine varnish. *J Dent Res* 70:1252-1257, 1991.
20. Emilson CG. Susceptibility of various microorganisms to chlorhexidine. *Scand J Dent Res* 85:255-265, 1977.
21. Sandham HJ, Brown J, Phillips HI, Chan KH: A preliminary report of long-term elimination of durable mutans Streptococci in man. *J Dent Res* 67:9-14, 1988.
22. Spets-Happonen S, Markkanen H, Pollanen L, Kauppinen T, Luoma H. Salivary Streptococcus mutans count and gingivitis in children after rinsing with a chlorhexidine-fluoride solution with and without strontium. *Scand J Dent Res* 93:329-335, 1985.
23. Unpublished data, personal communication, Gary Pitts, Castle Beach Co, Calif.
24. Loesche WJ, Eklund S, Earnest R, Burt B. Longitudinal investigation of bacteriology of human fissure decay: epidemiological studies in molars shortly after eruption. *Infect and Immun* 46(3):765-772, 1984.
25. Van Palenstein Helderman WH, Ijsseldijk M, Huis in't Veld JH. A selective medium for the two major subgroups of the bacterium Streptococcus mutans isolated from human dental plaque and saliva. *Archives of Oral Biology* 28(7):599-603, 1983.
26. Caufield PW, Cutter GR, Dasanayake AP. Initial acquisition of mutans Streptococci by infants. Evidence for a discrete window of infectivity. *J Dent Res* 72(1):37-45, 1993.
27. Hagan T, Shah GR, Caufield PW. DNA fingerprinting for studying transmission of Streptococcus mutans (abstract). *J Dent Res* 67:407, 1989.
28. Kulkarni GV, Chan KH, Sandham HJ. An investigation into the use of restriction endonuclease analysis for the study of transmission of mutans streptococci. *J Dent Res* 68(7):1155-1161 1989.
29. 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* 70(11):1401-1408, 1991.
30. Slots J, Taubman M. *Contemporary Oral Microbiology and Immunology*. Mosby Year Book, 1992.
31. Krasses B, Edwardsson L, Svensson I, Trell L. Implantation of caries-inducing streptococci in the human oral cavity. *Arch Oral Biol* 12:231-236, 1967.