Effect of chlorhexidine varnish mouthguards on the levels of selected oral microorganisms in pediatric patients

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Abstract

**Purpose:** The effect of a chlorhexidine varnish delivery system on the levels of selected oral microorganisms was evaluated in caries-active pediatric patients, ages 4 to 12 years old.

**Methods:** Forty-six patients were enrolled into the study when they had multiple carious surfaces and salivary mutans streptococci (MS) levels higher than 10^4 colony forming units (CFUs) per milliliter. This study incorporated a double-blind design and patients were randomly assigned to either the chlorhexidine treatment group or the placebo group. Complete-arch, vacuum-adapted mouthguards (0.02 in. polypropylene coping material) were custom fabricated and coated internally with either a 3.0% chlorhexidine varnish or a placebo varnish. Two pretreatment paraffin-stimulated saliva samples were obtained for culturing prior to varnish treatment. Saliva samples were also obtained immediately after treatment and once a month for up to three months after wearing the mouthguard appliances. Dental restorations were placed at most of these saliva collection visits. Mouthguards were reportedly worn for an average of 9.7 hours per night for approximately seven nights by 40 subjects (87%).

**Results:** After two months, and after three months, there was a significant reduction in MS levels immediately after the chlorhexidine varnish treatment. Total anaerobic and total facultative bacteria levels were not significantly affected.

**Conclusion:** One week of nightly use of the chlorhexidine varnish mouthguard system is effective at reducing the number of MS in caries-active pediatric patients in the mixed and primary dentition for at least three months. (Pediatr Dent 21:169–175, 1999)

It has been known for about 70 years that *S. mutans* is associated with dental caries infection. It was demonstrated that the initiation of dental caries was preceded by large numbers of *S. mutans* and that lactobacilli increased significantly only after the appearance of caries. However, caries frequently occurred in the absence of lactobacilli, but never in the absence of *S. mutans*. This indicated that *S. mutans* was required for the initiation of dental caries. Even though lactobacilli were not involved in the commencement of dental caries, it was shown that lactobacilli are contributors to the ongoing process after the establishment of caries.

Candida species may have the potential to be used as caries predictors. Candida counts were found to be significantly correlated with subsequent two-year caries increment. Correlations between Candida counts and caries increment were only slightly less than those for either lactobacilli or MS for caries increment. Also in older adults, the levels of yeast were significantly associated with decay in multivariate models. The association of yeast with caries may be related to the aciduric nature of *Candida albicans* and high numbers of *C. albicans* may merely reflect oral conditions which are favorable for caries development.

Bisbiguanide chlorhexidine is currently the most potent chemotherapeutic agent against *mutans* streptococci and dental caries. This chemotherapeutic agent has been shown to cause a decrease in dental caries activity among highly caries-active children and that decrease was associated with a significant reduction in MS numbers. It was demonstrated in 1972 that a 0.2% chlorhexidine solution had a caries-inhibiting effect on an in vivo caries model. Subsequent studies used a variety of methods for delivering chlorhexidine in solutions, gels, or pastes, but the results varied from no significant change in plaque or caries formation to moderate caries-inhibiting effects when compared to placebo treatment.
This probably reflected that the contact time of the MS to these chlorhexidine agents was too brief.

Friedman and Golomb demonstrated that it was possible to obtain sustained (slow) release of chlorhexidine for several months in vitro. Balanyk and Sandham developed a varnish vehicle that was safe in humans, compatible with chlorhexidine and able to release the chemotherapeutic agent over an extended period of time. This varnish released chlorhexidine into the oral environment at low but bactericidal levels for approximately two weeks. This sustained release chlorhexidine varnish was proven to be very successful at suppressing MS for prolonged periods and more effective than other chlorhexidine therapies. A single application of the chlorhexidine varnish to the teeth resulted in the elimination of detectable MS from the saliva of some individuals for many weeks. The chlorhexidine varnish, when applied before the placement of fixed orthodontic appliances, was able to significantly reduce the levels from baseline values for up to seven months. Another group, using another kind of vehicle varnish, sandarac resin, reported that MS was significantly suppressed for at least four weeks after a single chlorhexidine application and for some subjects the bacteria was effectively suppressed over six weeks. This group demonstrated that a chlorhexidine varnish in contact with the tooth surface for only 15 min was sufficient to achieve long-term suppression.

Another form of delivery of the chlorhexidine varnish was investigated using a coping-type mouthguard appliance to deliver the varnish. The chlorhexidine varnish was painted on the inner aspects of the mouthguard appliances instead of directly on the tooth surface, thus eliminating the laborious task of coating individual teeth. This system, used at night for one week, was successful at significantly suppressing MS levels for up to three months without retreatment.

The development of a sustained-released chlorhexidine varnish has made antimicrobial treatment against caries infection effective for extended periods of time. With the incorporation of a coping-type mouthguard appliance, the delivery of the chlorhexidine varnish is simplified and not time consuming for the dental practitioner and the dental patient. Past chlorhexidine varnish studies have predominantly observed only subjects with a permanent dentition who were at risk of necessarily being caries active.

The purpose of this clinical investigation was to study the effects chlorhexidine varnish mouthguard appliances would have on selected oral microorganisms in caries-active pediatric patients in the primary and mixed dentitions.

Methods

After approval was received from the Human Subjects Review Committee, patients from the University of Michigan graduate pediatric dental clinics, ages 4 to 12 years, were screened for elevated MS levels in their saliva. Those subjects having MS levels greater than 10^5 colony forming units, (CFU)/mL in paraffin-stimulated saliva, requiring more than three surfaces of dental restorations, demonstrating relatively cooperative behavior, not requiring antibiotic prophylaxis for invasive dental procedures, and having no medical contraindications qualified for participation in the study. Patients were randomly assigned to either the placebo group or the experimental group. The procedures, possible discomforts or risks, and possible benefits were explained fully to the human subjects involved and their parents, and their informed consent was obtained prior to the investigation. Parents/guardians were also informed of the financial incentive that would be distributed at two and three months after the use of the mouthguard appliances.

Maxillary and mandibular full-arch alginate impressions were taken for custom fabrication of the mouthguards. Polypropylene coping material (0.02-in. coping and temporary splint material; Buffalo Dental Manufacturing, Syosset, NY) was vacuum adapted to each cast with a vacuum adapter after three layers of die spacer (SPACE-IT; George Taub Products and Fusion Co., Inc., NJ) were painted on the crowns of the casts. The polypropylene shell was then trimmed approximately 1.0 mm apical to the free gingival margin. The inner aspect of both mouthguards was painted with three applications of one of the two varnishes prepared i.e., the placebo varnish or the chlorhexidine treatment varnish. The chlorhexidine varnish consisted of an ethanolic solution containing ethyl cellulose polymer and 3.0% chlorhexidine diacetate. The subsequent dry film of varnish was 30% by weight of chlorhexidine acetate. From a previous study, the average dose of chlorhexidine present in a pair of mouthguards was determined to be approximately 30 mg. The placebo varnish was identical to the chlorhexidine varnish but without the 3.0% chlorhexidine diacetate.

Saliva sampling was done at the beginning of all dental visits. Treated mouthguards were delivered immediately after procurement of the second saliva sample. This dental appointment usually coincided with the initiation of dental restorative treatment. The second saliva sample served as the baseline measurement. This study was designed as a double-blind experiment, in which samples were coded and study group identity was unknown at the time of microbiological counting. The patients were given written instructions to insert both the maxillary and mandibular mouthguards upon retiring to bed each evening, to wear them all night while sleeping, and to remove them upon arising in the morning. A daily log was handed out to record the amount of hours the appliance was worn per night.

After one week of wearing the mouthguard appliances, the patient was seen in order to retrieve the mouthguard appliances and to obtain a third saliva sample. A questionnaire was completed by the parent to determine the number of nights and the hours per night the patients wore the appliance. Also the extent to which they experienced an objectionable taste, alterations to the taste of their food, or burning sensations in the mouth was elicited. Subsequent saliva samples were obtained at one, two, and three months after retrieval of the mouthguard appliances. These saliva-collection appointments corresponded to the patient’s dental restorative appointments. The mouthguard appliances were delivered prior to the completion of all restorative work. Thus, all subjects were wearing the mouthguard appliances in the presence of frank carious lesions.

A paraffin-stimulated saliva sample was obtained from the patients on multiple occasions. The patient expectorated for three minutes into a plastic disposable funnel and the saliva was collected in a disposable plastic tube (Cyrorgenic Vial #25708-5, Corning Inc., Corning, NY). Samples were stored in liquid nitrogen for approximately one to four weeks until cultured. For processing, 1.0 mL of saliva was immediately added to 9.0 mL of reduced transport fluid to achieve a 1:10 dilution.
Samples were serially diluted in reduced transport fluid and various culture media were inoculated using a spiral plater (Spiral Systems, Cincinnati, OH). The culture media used were tryptone-yeast extract-cystine-sucrose-bacitracin agar (TYCSB) for MS,36 enriched trypticase soy agar (ETSァ) for anaerobic organisms,37 ESTA with metronidazole (Flagyl) for total facultative anaerobic organisms,38 Rogosa lactobacillus selective medium agar (LBS) for lactobacilli,39 and Sabouraud medium for yeast.

All inoculated plates, except the Sabouraud, were transferred to an anaerobic chamber (Coy Laboratory, Ann Arbor, MI) of 85% N2, 10% H2, and 5% CO2 and incubated at 35°C for five to seven days. CFUs were enumerated under a stereomicroscope by the sector count method.39 The Sabouraud plates were incubated at 37°C at room atmosphere for two days. CFUs per unit volume were transformed to log10 counts to control variance for the purpose of applying parametric statistical tests to the data. PROC MIXED in SAS/STAT® (SAS/STAT® software, 1996, SAS Institute, Cary, NC) was used to carry out a repeated-measures ANOVA on the ratio of log10 counts to baseline log10 counts. In these analyses, tests for groups, time and group by time interaction were obtained. The significance of differences in log10 counts among saliva-collection visits were tested with univariate repeated-measures ANOVA, and pairwise comparisons were made between baseline values and the four after-treatment values with paired t-tests.

Results

A total of 46 patients were recruited and 40 reported usage of the mouthguard appliances. Three patients were lost from the study due to poor compliance in showing for their scheduled dental appointments. Another three patients refused to wear the mouthguard appliances after experiencing the first restorative dental appointment and were very uncooperative for all of their dental treatment. The average age of the 40 patients was 8 years (median=7.8) with a range from 4.4 to 12 years. The mean number of carious surfaces was 16.4 (median=15) with a range from 3 to 42 surfaces. For the 40 patients who wore the mouthguard appliances, 18 patients (45%) were in the chlorhexidine treatment group and 22 (55%) were in the placebo group. When the patients’ baseline measurements of age, gender, race, and the number of carious surfaces and the microorganism levels were compared, there were no statistical differences between the chlorhexidine and the placebo groups.

When the patients’ baseline demographics and the number of carious surfaces were compared, there were no statistical differences between the chlorhexidine treatment group and the placebo group (P=0.190) before the delivery of the mouthguard appliances. The gender distribution was similar between the groups (P=0.381) with 44% male and 56% female in the chlorhexidine group and 55% male and 46% female in the placebo group. The ages of the subjects in both groups were comparable (P=0.111). The mean age was 7.4 years (median=7.1 years) with a range from 4.4 to 12.0 years for the chlorhexidine group and 8.7 years (median=8.9 years) with a range from 5.1 to 12.0 years for the placebo group. The number of carious surfaces and the restorative needs were also similar between the groups (P=0.856). There was a mean of 17 carious surfaces (median=16) with a range from 5 to 36 carious surfaces in the chlorhexidine group and a mean of 16.3 carious surfaces (median=15) with a range from 3 to 42 carious surfaces in the placebo group. There was a noticeable difference in the race distribution between the groups however there were no statistical difference (P=0.215). In the chlorhexidine treatment group, 15 (83%) of the patients were African-American and two (11%) were Caucasian, whereas in the placebo group there was an equal representation of both races—ten each (46%).

The questionnaire was filled out with the help of parents or guardians by all 40 patients who reported that they wore the appliances. The average number of nights the mouthguards were worn was 6.7 nights with a range from five to eight nights with a mean wearing time of 9.6 hours per night. Seven (18%) of the patients thought that the mouthguards were uncomfortable. Only four (10%) of the patients claimed that the mouthguards “sometimes kept them up at night”. Thirteen (33%) of the patients complained that the mouthguards had a bad taste, nine of whom were in the chlorhexidine group. However, these patients still wore their mouthguard appliances at night. Three (8%) of the patients claimed that the mouthguards changed the taste of their foods, two of whom were in the chlorhexidine group. Thirty-nine (98%) patients reported that the mouthguards did not cause the tongue to “burn”. Parent and patient acceptance of the mouthguards was favorable as determined from the responses in the questionnaire. When the questionnaire information was considered separately for the control and the experimental group, there was no statistical difference between the groups.

There was no statistical significant difference between the baseline saliva levels of MS, lactobacilli, yeast, total anaerobic bacteria, and total facultative bacteria for the chlorhexidine and the placebo groups. The baseline saliva levels for the chlorhexidine treatment group and the placebo group for MS were 7.413 x10⁸ vs. 7.285 x10⁸ CFU/mL, respectively, (P=0.473), for lactobacilli were 7.413 vs. 11297 (P=0.758), for yeast were 399 vs. 481 (P=0.414), for total anaerobic bacteria were 1.400 x 10⁹ vs. 1.377 x 10⁹ (P=0.222), and for total facultative bacteria were 1.122 x10⁸ vs 1.100 x10⁸ (P=0.295).

When compared to baseline levels, there was a statistically significant reduction of MS immediately after treatment
The placebo group, there was a parallel reduction of yeast lev-

time interaction for this type of oral microorganisms. There was
demonstrated a pattern of MS reduction over time which par-
when compared to baseline levels, there was no statistically
significant reduction in the levels of total anaerobic bacteria
against time and of total facultative bacteria against time re-
be completed. There was also no statistically significant reduction of
these oral microorganisms at one, two, and three months after
the chlorhexidine treatment. When the placebo group was
compared with the chlorhexidine group, the average ratios of
log_{10} counts to baseline log_{10} counts of total anaerobic bacteria
against time and of total facultative bacteria against time re-
vealed that there was a group by time interaction for these types
of oral microorganisms. There was no statistical significant differ-
ence between the chlorhexidine group and the placebo group
for the total anaerobic bacteria (P=0.826) levels and for the total
facultative bacteria (P=0.617) levels after the retrieval of the
mouthguard appliances.

Discussion

Dental caries has a multifactorial etiology. Most preventive
measures involve sealants,40 fluoride,41,42 sugar reduction,43 and
sugar substitutes,44 each targeting a different factor necessary
for the formation of dental caries. However, none of them
specifically target the chief pathogen responsible for dental
caries—MS. In spite of these effective preventive methods,
dental caries is still the most common infectious disease of
childhood14 and new methods of diagnosing and treating
decay, especially in the primary dentition, need to be de-
veloped. This study demonstrated that the number of MS can be
significantly reduced when a chlorhexidine varnish is applied
to mouthguard appliances that are worn for seven consecutive
nights by high caries-active pediatric patients in the primary
and mixed dentition.

The use of the chlorhexidine varnish has been successful in
high caries-risk adults. However, there are no reported studies
using pediatric patients who are in the mixed or primary den-
tition. Also, previous studies did not involve patients with the
high caries activity of the present subjects, and if dental resto-
ration was necessary, placement was always prior to
antimicrobial treatment. This study evaluated the effects of
chlorhexidine varnish mouthguards on pediatric patients with
open carious lesions. These pediatric patients received the
chlorhexidine treatment before all their dental restorative treat-
ment was completed.

One concern with chlorhexidine treatment of caries-active
individuals is that of superinfection.45 Chlorhexidine is a po-
tent broad-spectrum surface disinfectant. However, it does not
penetrate well into retentive areas such as carious dentin. Af-
ter treatment with chlorhexidine at therapeutic levels, those
microorganisms which survive in large numbers will have some
advantage at recolonizing the tooth surfaces. As such, the load
of MS on the dentition could actually increase subsequent to
chlorhexidine treatment if open carious lesions are present. The
long-term suppression of MS levels on the teeth of the pediat-
ric subjects observed in this study suggests that superinfection
is not a common sequelae to chlorhexidine suppression with frank carious lesions present.

Because we show that the chlorhexidine-varnish mouthguard appliances can significantly reduce MS levels for at least three months in caries-active pediatric patients, the data argue that this treatment can be delivered on the same appointments when dental restorations are being placed. This would be more cost-effective, as extra visits for the delivery of the chlorhexidine varnish mouthguard appliances would be eliminated. Dental chair time would be reduced and the parents and patients would not have to return to the dental office as often, thereby increasing efficiency and cost-effectiveness.

The chlorhexidine varnish used by Sandham et al.25, 26 and Schaeken and de Hann28 is painted directly on each tooth, and this procedure requires a considerable amount of time. Another inherent problem with this approach is that the chlorhexidine varnish has to be applied to a dry, isolated, plaque-free surface. The use of the mouthguard appliances for the chlorhexidine delivery minimizes the amount of time the dental patient has to stay in the dental chair. This may be advantageous in restless and anxious pediatric dental patients, and may offer a more effective method to suppress MS levels in pediatric patients. If there should be any adverse effects, such as a bad taste, the chlorhexidine varnish can be conveniently removed from the oral cavity.

An experimental and a placebo group were used in this study. Each patient acted as their own control as measured from the first two saliva samples. The placebo group was essentially a mouthguard control group which enabled us to evaluate any effects on the microorganisms that may have occurred from placing dental restorations, from wearing the mouthguard appliance, and from the ethyl cellulose varnish with no chlorhexidine. An argument can be made that the mouthguard appliance itself, with no antimicrobial agent but covering the teeth, would block the beneficial effects of saliva. However, the time frame in which the placebo appliance was worn appears to have been too short to have caused any adverse effects.

Patient compliance and parental support is important for this delivery system to work. The decline in MS levels was the most objective of compliance indicators and did indicate favorable patient compliance. However, positive compliance was not as strongly reflected by the number of broken/canceled appointments. In order to curb this situation, parent and patient behaviors were modified when appropriate. They were educated on the importance of improving and maintaining good oral health. Patients were given printed daily log sheets with instructions to encourage wearing the appliances. The questionnaire was filled out, with the help of parents or guardians, by all of the patients. Most patients reported compliance in wearing the mouthguards as instructed and reported no major discomfort in wearing the appliances. The compliance and acceptance of chlorhexidine-varnish mouthguard appliances used in this study correlated well with those of Hildebrandt et al.32 and Sandham.46

There was a significant reduction in the number of MS immediately after the chlorhexidine varnish treatment that lasted for up to three months after treatment. These findings are similar to those observed in adults using identical mouthguard appliances.32 In that adult study there was a greater reduction of MS levels immediately after and at one month after the chlorhexidine varnish treatment. The reason for the differences between these two studies for MS levels before the three-month check may have been the presence of open carious lesions. In this study, chlorhexidine varnish treatment was rendered before all dental carious lesions were restored. Hildebrandt and coauthors32 first restored all of the carious lesions and then delivered the chlorhexidine-varnish mouthguard appliances. All foci of infection were brought under control before using the chlorhexidine varnishes in order to optimize the antimicrobial affects. Because this was not done in our study, the MS levels were not significantly reduced at the one-month mark, yet there was a statistically significant reduction immediately after, as well as at two and three months after the chlorhexidine treatment. The significant one log (90%) reduction of MS levels after the chlorhexidine varnish use in the presence of open carious lesions demonstrate the efficacy of the chlorhexidine varnish.

Restoration of open carious lesions removes retentive sites harboring MS from the dentition. Loesche et al.35 showed that the effect of restoring open carious lesions on salivary MS levels was temporary, the reductions lasting less than a week. In a previous study,34 no significant change in MS levels one to two weeks following restoration of all open carious lesions was observed. It is tempting to attribute the reduction in MS levels seen over time in both the experimental and placebo group to the effect of restoring frank carious lesions. However, the magnitude of the decline was small, particularly in the placebo group. Because a cohort was chosen biased in favor of disease (MS levels greater than 10 CFU/ml), the trend towards greater differences between MS levels in samples compared to baseline levels over time may be artifactual, caused by regression towards the mean.

Lactobacilli levels did not appear to be affected by chlorhexidine treatment as has been reported in other studies.32, 34 There was an observable reduction immediately after and one month after wearing the mouthguards in both the placebo group and the chlorhexidine treatment group. These decreases in lactobacilli levels can be attributed to the gradual restoration of the carious lesions.

The yeast levels were not significantly affected by the chlorhexidine treatment. The reduction in yeast levels in both the chlorhexidine and the placebo groups can be attributed to...
the gradual reduction of carious lesions. This was similar to that reported by Pienihäkkinen and Russel et al., where salivary yeast was one of the predictor variables for caries experience.

The total facultative bacteria and the total anaerobic bacteria levels were unaffected by the chlorhexidine varnish treatment and these levels remained stable throughout the study as has been reported by others. This is an attractive outcome of the chlorhexidine treatment. The lack of change in total facultative bacteria and the total anaerobic bacteria levels indicates that this experimental treatment is fairly specific for MS. A reduction in MS levels in the face of stable total facultative bacteria and the total anaerobic bacteria levels shows that MS is being selectively suppressed. Not only are MS levels being suppressed but MS proportions with respect to the background microorganisms are being reduced.

The chlorhexidine-varnish mouthguard appliances exerted its most profound effect on MS. A contributing factor to the selective reduction of MS is the direct targeting of the chlorhexidine to the teeth, which is the primary habitat of MS. Other microorganisms in the saliva which originate from other parts of the oral cavity are not significantly affected. Lactobacilli were not significantly affected probably because they are usually concentrated within a carious lesion and not on the tooth surface. The chlorhexidine is unable to penetrate into the carious lesion as it is retained to the negatively charged tooth surface where MS can be found. Thus, one can conclude that MS are more sensitive to chlorhexidine-varnish mouthguard appliances than lactobacilli because of their location in the plaque.

Chlorhexidine rinses have several disadvantages such as reduction of nonpathogenic as well as pathogenic flora, staining of teeth, disturbances of taste, increased calculus formation, minor irritations, and superficial desquamation of the oral mucosa. No serious side effects have been observed with the use of chlorhexidine in the form of a varnish. No tooth stains or calculus formation resulted as determined by visual clinical examination. The reduction of these negative effects of chlorhexidine can be attributed to the infrequency of the varnish application compared to other chlorhexidine treatment forms that use multiple applications. The possibility of chlorhexidine ingestion would be substantially decreased when used in the form of a varnish. Also children would be exposed to reduced absolute amounts of the varnish, as their dentition is smaller than adults.

The development of a sustained-released chlorhexidine varnish has made antimicrobial treatment against caries infection effective for extended periods of time. With the incorporation of a coping-type mouthguard appliance, the delivery of the chlorhexidine varnish is simplified and not time consuming for the dental practitioner and the dental patient. Chlorhexidine rinses such as Periex® (Procter & Gamble, Cincinnati, OH) and Perioguard® (Colgate-Palmolive Company, Canton, MA) are currently used for adults in the United States. The clinical effectiveness and safety of these rinses have not been established in children younger than 18 years of age, although clinical use in children is common. The chlorhexidine varnish is currently being used to treat patients in Europe and in Canada. U.S. Food and Drug Administration approval is currently being requested for two chlorhexidine varnish systems for use in the US. These are Chlorzoin® (Knowel Therapeutic, Canada) and Cervitec® (Viadent, Orion Diagnostica, Finland).

The long-term suppression of MS caused by the chlorhexidine varnish seems to be a selective process. The total anaerobic bacteria and total facultative bacteria levels were not affected and there was no significant reduction of yeast and lactobacilli attributed to the chlorhexidine treatment. This occurred because MS was the most sensitive to the chlorhexidine treatment of all the oral microorganisms studied.

Conclusions

1. One week, night-time only use of a chlorhexidine-varnish mouthguard system is effective at reducing the number of MS in caries-active pediatric patients in the mixed and primary dentition for at least three months.
2. The chlorhexidine-varnish mouthguard system is effective at reducing the number of MS in pediatric patients even when used before all dental caries are restored or removed.
3. The chlorhexidine-varnish mouthguard system appears to selectively suppress MS as there were no significant effect on yeast, lactobacilli, total anaerobic bacteria, and total facultative bacteria levels.
4. Overall, parents and patients were receptive of the chlorhexidine-varnish mouthguard system and compliance in wearing the appliances was favorable.
5. No major adverse effect of the chlorhexidine-varnish mouthguard system was reported.

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