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What is This?
Recolonization of Human Tooth Surfaces by *Streptococcus mutans* after Suppression by Chlorhexidine Treatment

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In eight subjects who were initially highly colonized with *Streptococcus mutans* and who used a 1% chlorhexidine gel, the numbers of this organism were suppressed in both plaque and saliva. Bacterial plaque samples were obtained from all tooth surfaces, and the colonization pattern of *S. mutans* was studied over a 26-week period. At baseline, 83% of all surfaces harbored *S. mutans* with buccal surfaces colonized in higher frequency than the others. After chlorhexidine treatment, the proportion of tooth surfaces colonized by *S. mutans* was reduced to a low level. Re-appearance was slow. *S. mutans* was first recovered from the most posterior teeth in the mouth, the molar surfaces were recolonized earlier than were those of pre-molars and anterior teeth, and the buccal surfaces were recolonized more readily than were other tooth surfaces. The data show that there is a specific recolonization pattern of *S. mutans* after chlorhexidine treatment, and that the re-emergence of *S. mutans* is most probably due to regrowth of bacteria which have not been eradicated.


**Introduction.**

*Streptococcus mutans* is more sensitive to chlorhexidine than are many other oral micro-organisms (Emilson, 1977) and is specifically suppressed after intensive treatment with chlorhexidine gel. These findings have been applied for reduction of dental caries in schoolchildren (Zickert et al., 1982). However, *S. mutans* gradually re-appears and generally reaches pre-treatment levels two to six months after chlorhexidine gel treatment (Emilson, 1981; Maltz et al., 1981). It is well known that various factors — such as variations in dietary carbohydrates, indigenous microflora, or in properties of saliva — can influence the colonization and intra-oral spread of *S. mutans* (Krasse et al., 1967; Van Houte, 1980; Van der Hoeven, 1980; Svanberg and Krasse, 1981), and for re-appearance of *S. mutans* after chlorhexidine treatment, some of these factors might also be important. In the search for more effective methods leading to a prolonged reduction of the *S. mutans* population, we need a better understanding of the re-appearance pattern of *S. mutans* and the mechanisms involved. The purpose of this study was to investigate further the recolonization of *S. mutans* in the dentition after a short-term application of a chlorhexidine gel.

**Materials and methods.**

**Subjects.** — Eight males and females, ranging in age from 22 to 32 years, gave their informed consent to participate in the study. Each had a full dentition, and there was a mean dental caries experience of 34 decayed, missing, and filled surfaces, with a range of from six to 89. None of the subjects had detectable frank caries lesions or defective restorations. Filled approximal tooth surfaces were re-contoured and polished in order to facilitate the penetration of the gel into the interproximal space.

**Sample collection.** — Before chlorhexidine treatment, saliva and plaque samples were collected to establish baseline microbial data for *S. mutans*. After-treatment samples were taken at regular intervals for 26 weeks. Saliva and plaque were collected in the mid-morning, from two to three hours after breakfast. Before each sampling session, the subjects had refrained from toothbrushing for 24 hrs. Whole stimulated saliva was obtained by paraffin-chewing, and 1 mL was transferred to VMG II transport medium (Möller, 1966). Plaque samples were taken from all tooth surfaces in the dentition. Immediately before plaque sampling, the subjects were asked to rinse their mouths with water. The teeth were then isolated with cotton rolls and gently dried with compressed air. Plaque was collected from the cervical halves of buccal and lingual tooth surfaces and along the entire fissure or margin of each restoration, on the occlusal surface, by means of the tips of sterile wooden triangular toothpicks (TePe, Eklund & Pettersson AB, Malmö, Sweden). The tips with plaque were cut off and dropped into wells in a microtiter-plate containing 0.3 mL of reduced transport fluid (RTF; Syed and Loesche, 1974). Plaque samples from the approximal tooth surfaces were taken with a triangular toothpick, as described by Kristofferson and Bratthall (1982). The toothpick was inserted into each interproximal space. Both sides of the toothpick, representing the mesial and distal surfaces of each interproximal space, were then immediately pressed against the surface of *S. mutans*-selective mitis salivarius bacitracin (MSB) agar (Gold et al., 1973) in contact Petri dishes (Nunc, Roskilde, Denmark). Plaque samples from the tongue were also taken after treatment. The tongue was first gently dried with a sterile cotton roll, and then material on the surface and in the crypts of the tongue was collected by wooden toothpicks scraped three times repeatedly over the same area of 0.5 cm² on the central part of the dorsum. The material collected was transferred to RTF medium. All saliva and plaque samples were cultured within three hours of collection.

**Bacteriological procedures.** — The plaque samples were sonically dispersed for 10 sec at 60 W power by means of a Branson sonifier W185 equipped with a microtip. Aliquots of 25 µL were plated in duplicate on MSB agar. The saliva samples were dispersed on a Whirlimixer for 30 sec, and from appropriate dilutions in 0.05 mol/L phosphate buffer (pH 7.3), 25-µL portions were plated in duplicate on MSB agar. The inoculated plates were incubated at 37°C in 5% CO₂ in N₂ for two days. *S. mutans* was enumerated on the MSB agar plates by its typical colonial morphology (Krasse, 1966; Emilson, 1983), confirmed, when necessary, with serotyping by immunofluorescent identification (Bratthall, 1972). The number of *S. mutans* colony-forming units (CFU) per mL saliva was estimated. The presence of *S. mutans* was determined for each tooth surface and tongue sample, and the number of colonies was counted and expressed in terms varying from 0 to 4, as follows: score 0 = <5 CFU; 1 = 5-10 CFU; 2 = 11-100 CFU; 3 = 101-400 CFU; and 4 = >400 CFU. The lowest limit of 5 CFU for positive detection was used in order to...
avoid or limit the possibility that the undiluted plaque samples would be scored as positive due to contamination with S. mutans-infected saliva. The scores for mesial and distal surfaces in each interproximal space were the same in 82% of all samples. The mean score value of the two surfaces was therefore calculated and considered to be the degree of infection for the approximal surfaces.

Treatment procedures. — After the bacteriologic baseline data had been obtained, each subject received professional tooth cleaning with rubber cup and pumice, and interproximal cleaning with unwaxed dental floss. The participants were given individually designed maxillary and mandibular polystyrol applicators and instruction/demonstration of how to use the gel at home. A 1% chlorhexidine digluconate (ICI Ltd., Macclesfield, England) gel (pH 7.2) containing 2% methylcellulose and flavoring agents was placed in each applicator and applied to the teeth three times for five min, with intervals of five min between each application, during days 1, 2, 10, and 11, at the clinic. During days 3 to 9, the subjects used the gel at home once a day for five min after their usual toothbrushing. Interproximal cleaning with toothpicks was added to the oral hygiene procedures, if not regularly used before, in order to facilitate the access of the gel to these sites. No other change in oral hygiene practice was instituted, nor were any dietary restrictions imposed. The subjects were instructed not to rinse their mouths or eat for at least 30 min after each gel treatment.

Statistics. — Statistical comparisons of changes in the levels of S. mutans over time were performed by means of the non-parametric sign test. Computations were based on intensity measures of prevalence of S. mutans for each individual at each examination.

Results.

Prior to treatment, all subjects showed counts of S. mutans in excess of \(10^6\) CFU per mL saliva. The number ranged from \(0.1 \times 10^6\) to \(3.4 \times 10^6\) S. mutans CFU per mL (median value 1.2 \(\times 10^6\)).

The proportion of tooth surfaces colonized by S. mutans at baseline in the eight subjects is illustrated in Fig. 1. Of the total number of 1061 tooth surfaces, 83.1% were positive for S. mutans. The buccal surfaces were colonized in a higher frequency than were the other surfaces and showed a presence of S. mutans on 91% of the sites, as compared with 84% for approximal, 80% for lingual, and 70% for occlusal surfaces. The proportion of S. mutans-positive surfaces heavily infected (scores 3 and 4) ranged between a third for lingual and approximal surfaces to almost half for buccal and occlusal surfaces.

Four days after the 11-day chlorhexidine treatment period, the proportion of tooth surfaces with 1, 2, 3, 4 scores was reduced to 2.0%, as compared with 83% at baseline (Table). S. mutans was not detected in two subjects. Four subjects had one or two surfaces with S. mutans, while, in two subjects, four and 11 tooth surfaces were still colonized by S. mutans. In the six subjects with S. mutans, 16 of the 21 infected sites were found on 2nd and 3rd molars in plaque from three approximal spaces and four buccal, four lingual, and two occlusal surfaces. Of the remaining five infected tooth surfaces, three were found in plaque samples from around an ill-fitting gold crown on a canine and two in plaque from a second pre-molar and a first molar. The population of S. mutans in saliva, represented by the median number, was reduced from a level of 1.2 \(\times 10^6\) CFU per mL (range 0.1–3.4 \(\times 10^6\)) before treatment to less than \(10^2\) CFU per mL, the limit of detection of the organism with the dilutions used. S. mutans was not detected in saliva in four of the subjects, and counts of \(12 \times 10^3\) or less per mL were found in the other subjects.

The recolonization of the tooth surfaces by S. mutans and its detection in saliva are illustrated in Fig. 2. After the chlorhexidine treatment, S. mutans gradually recolonized, and after 10–12 weeks about 60% of all tooth surfaces were again colonized by S. mutans. After 26 weeks, 67% of the tooth surfaces were colonized by S. mutans, and the percentage of

![TOOTH SURFACES](image)

**TABLE**

DISTRIBUTION OF S. mutans SCORE ON 1061 TOOTH SURFACES BEFORE, AND FOUR DAYS AFTER, CHLORHEXIDINE (CH) TREATMENT

<table>
<thead>
<tr>
<th>S. mutans Score</th>
<th>Total Surfaces</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before CH</td>
</tr>
<tr>
<td>0</td>
<td>179</td>
</tr>
<tr>
<td>1</td>
<td>213</td>
</tr>
<tr>
<td>2</td>
<td>324</td>
</tr>
<tr>
<td>3</td>
<td>130</td>
</tr>
<tr>
<td>4</td>
<td>215</td>
</tr>
</tbody>
</table>

| surfaces colonized highly | 882 | 83.1 | 21 | 2.0 |
| colonized (scores of 3 and 4) | 345 | 32.6 | 2 | 0.1 |
surfaces with bacterial scores of 3 and 4 was almost similar to that observed at baseline. Compared with baseline level, the reduction in the numbers of surfaces infected with S. mutans was still statistically significant after 26 weeks (p<0.01). About 6% of the colonized tooth surfaces at week 26 were surfaces where S. mutans was not recovered at baseline. None of them showed S. mutans score 3 or 4. When compared with recolonization of the tooth surfaces, the re-appearance of S. mutans in saliva was proportionally slower at first, but after 12 weeks the S. mutans population increased markedly.

Fig. 3 illustrates the recolonization of different tooth surfaces by S. mutans over the 26-week post-treatment period. The buccal surfaces were more readily recolonized than were other tooth surfaces. After 10 weeks, the proportion of buccal surfaces with S. mutans had reached about 80% of the baseline level. The re-appearance of S. mutans on the other surfaces was slower. The recolonization curves for approximal and lingual tooth surfaces were almost similar. After 26 weeks, the colonization level on these surfaces was 33% lower than that before treatment. The S. mutans level on occlusal surfaces had reached only about 50% of pre-treatment level after 10 weeks and 66% after 26 weeks.

Fig. 4 shows the recolonization pattern by S. mutans in the molar, pre-molar, and anterior regions. As noted earlier, the molars were hardest to disinfect. On these teeth, S. mutans was detected earlier than on pre-molars and on incisors and canines. After six weeks, 50% of the molars were recolonized, and at 12 and 26 weeks the percentage of molars with S. mutans was almost back to pre-treatment level. The recolonization by S. mutans was slower on the pre-molars but especially in the front region, where an effect of treatment was still apparent after 26 weeks.

In Fig. 5, the pattern of recolonization by S. mutans after chlorhexidine treatment is illustrated for individual teeth. Recolonization was most rapid on third molars, which were almost all colonized by S. mutans after 10 weeks. The other teeth became recolonized in successive order from second molars to pre-molars and, last, to incisors. The incisors were slowly recolonized by S. mutans, and 26 weeks after the antimicrobial treatment, S. mutans was detected on 28% of first incisors, as compared with 78% at baseline.
There were no significant differences in recolonization rate of *S. mutans* between left and right sides of the mouth during the experimental period. No difference in colonization level was found between upper and lower anterior teeth except at weeks 8 and 12, when upper second incisors had a higher degree of infection than did lower second incisors (p<0.05).

Fig. 6 shows the median *S. mutans* score in samples from tongue scrapings. No *S. mutans* colonies were detected during the first four weeks after chlorhexidine treatment. On the following sampling occasions, *S. mutans* was recovered and then in most samples in higher numbers in the first superficial scraping than in the next two scrapings on the same area of the dorsum of the tongue.

**Discussion.**

Short-term intensive chlorhexidine gel treatment caused a significant and long-lasting reduction in the population of *S. mutans* in subjects highly infected with the organism. Four days after termination of the chlorhexidine treatment, *S. mutans* was recovered from only a few tooth surfaces, as compared with 83% of all surfaces prior to treatment. The effect was also evident in saliva samples, which showed the presence of *S. mutans* in only four of the subjects and then in low numbers. The considerable reduction in the *S. mutans* population after chlorhexidine gel treatment is in accordance with earlier observations in humans (Emilson et al., 1976; Emilson, 1981; Maltz et al., 1981; Zickert et al., 1982; Schaeken et al., 1984, 1986).

The chlorhexidine treatment not only resulted in very few surfaces still harboring *S. mutans*, but also the level of *S. mutans* CFU in plaque from the infected surfaces was decreased. The results also show that despite the intensive antimicrobial treatment, once *S. mutans* has been established in the oral cavity, it is not easily eliminated. However, the data of Sandham et al. (1985) suggest that higher doses and a longer time of administration of chlorhexidine therapy would even further increase the numbers of tooth surfaces becoming free of *S. mutans*.

The few tooth surfaces that yielded *S. mutans* after treatment were mostly found on second and third molars and were evenly distributed among the different surfaces of these teeth. This observation shows that the most posterior teeth in the mouth are the ones that are most difficult to disinfect and where traces of *S. mutans* are most likely to be found when the eradication of the organism has not been total. These posterior surfaces were also those most heavily infected before treatment, an observation which is in line with other studies showing the distribution of *S. mutans* at different locations within the dentition (Keene et al., 1981; Kristoffersson et al., 1984; Scheie et al., 1984a).

Following chlorhexidine treatment, *S. mutans* gradually recolonized but needed a considerable time for re-appearance. Surfaces which became positive for *S. mutans* also became increasingly colonized over time. The number of colonized tooth surfaces remained significantly below original levels for more than 26 weeks following treatment. This observation confirms and extends earlier observations of chlorhexidine gel treatment in humans (Emilson, 1981; Maltz et al., 1981; Zickert et al., 1982). Similar findings have also been reported after iodine treatment (Gibbons et al., 1974; Caufield and Gibbons, 1979; Schaeken et al., 1984). In chlorhexidine studies where a 99.9% reduction of the *S. mutans* population has been obtained (Emilson, 1981), recolonization has been much slower than when only a partial removal of the organisms has been achieved (Kristoffersson et al., 1984; Schaeken et al., 1984, 1986). The observation that total cultivable count in saliva was at the same time reduced by 69% (Emilson, 1981) suggests that the chlorhexidine treatment selectively suppressed and af-
fected the colonization by \textit{S. mutans} more than that of many other micro-organisms. Since other members of the oral microflora, especially \textit{S. sanguis}, seem to be less affected and to re-establish much faster than \textit{S. mutans} after chlorhexidine treatment (Emilson, 1981; Schaeken et al., 1984), it is possible that they may retard the re-appearance of \textit{S. mutans} by interference (Van der Hoeven, 1980) and be partly responsible for the slow recolonization.

The present study also shows that re-appearance of \textit{S. mutans} in the dentition after chlorhexidine treatment followed a specific colonization pattern. \textit{S. mutans} was first recovered from the most posterior teeth in the mouth, and the molars were also recolonized earlier than were the pre-molars and anterior teeth. Whereas almost all third molars were infected ten weeks after therapy, the recolonization was notably slow on first incisors, and 26 weeks after the antimicrobial treatment, \textit{S. mutans} was recovered from only 28% of incisors, as compared with 78% at baseline. This finding indicates that the conditions for \textit{S. mutans} to establish and colonize are more favorable for molar teeth than for the other teeth in the mouth.

The buccal tooth surfaces were more readily recolonized than were the other surfaces, an observation that was unexpected. It had been anticipated that recovery of \textit{S. mutans} on the occlusal surfaces would occur earlier, since fissures can serve as a reservoir for \textit{S. mutans}, and it is difficult to reduce the population of \textit{S. mutans} in deeper parts of artificial fissures by attempts to suppress the organism (Swannberg and Loesche, 1977). However, whereas buccal surfaces were highly recolonized at 10 weeks following treatment, the presence of \textit{S. mutans} on the occlusal surfaces had reached only about 50% of the pre-treatment level. It is conceivable that the fast recolonization by \textit{S. mutans} of the buccal surfaces is related to suppression of a competitive microflora (Schaeken et al., 1986), since these surfaces in general are the easiest to clean by ordinary oral hygiene procedures.

The estimation of the prevalence of \textit{S. mutans} was based on the method of using sharp toothpicks to sample the surfaces. This method, which is rapid and permits many surfaces to be sampled, has shown a good reproducibility in repeated samplings of approximal tooth surfaces (Kristoffersson et al., 1982). In view of the fact that the method is a way of estimating the presence of \textit{S. mutans} in plaque, the data showed a remarkably low variation in recovery over the experimental period. Surfaces which became recolonized showed thereafter a highly consistent presence of \textit{S. mutans} with a steady, slow increase in infection score, thus demonstrating the validity of the sampling method for quantifying the \textit{S. mutans} load in the mouth.

The re-appearance of \textit{S. mutans} was proportionally slower in saliva than on the teeth. The salivary levels may, therefore, somewhat underestimate the presence of \textit{S. mutans} in the dentition during the weeks directly after antimicrobial therapy. This was noted in two of the subjects, where the organism was not detected in saliva four days after treatment, while a few tooth surfaces still harbored \textit{S. mutans}. This latter observation has also been noted in other studies (Kristoffersson and Bratt- hall, 1982).

In the present study, re-appearance of \textit{S. mutans} on the tongue occurred somewhat later than on the teeth and in the saliva. This finding shows that the prevalence of \textit{S. mutans} in saliva may also influence the presence of the organism on the tongue and contribute to the presence of \textit{S. mutans} on this site. The higher prevalence of \textit{S. mutans} in tongue samples from the first scraping than in the following scraping samples also supports this view. The observations also indicate that the sur-

Since dental caries is believed to be caused by specific bacterial species, notably \textit{S. mutans} (Hamada and Slade, 1980; Emilson and Krasse, 1985), treatment should be directed toward eradication or reduction of the pathogens. In order for \textit{S. mutans} colonization to be managed effectively, elimination of apparent retention sites (such as caries lesions and defective fillings) before antimicrobial therapy seems important, since restorative caries treatment has been reported to reduce the plaque level of \textit{S. mutans} (Keene et al., 1976; Scheie et al., 1984b). This was also illustrated by the persistence of \textit{S. mutans} in plaque samples from an ill-fitting gold crown in one of the treated subjects. The difficulty of disinfecting third molars and the rapid re-infection of these teeth also indicate the possibility of extraction of third molars in certain cases with severe caries problems, since this would apparently reduce the spread of \textit{S. mutans} to other teeth.

No attempts were made in this study to alter the dietary habits of the subjects. Since the level of \textit{S. mutans} is dependent on sucrose intake (Van Houte et al., 1976; Tanzer, 1979; de Stoppleaar et al., 1970), it is likely that dietary counseling aimed at a reduction of sucrose intake could have extended the time of \textit{S. mutans} suppression. This possibility is substantiated by observations in hamsters that the low proportion of \textit{S. mutans} in plaque achieved by chlorhexidine treatment persists when they are given a sucrose-free diet (Emilson and Westergren, 1979).

The observation that \textit{S. mutans} is first recovered from the most posterior surfaces in the dentition and then re-appears on the other teeth in a posterior-anterior direction is an indication that the occurrence of \textit{S. mutans} is due to regrowth of bacteria which have not been totally eliminated. In the subjects with a few infected posterior sites after treatment, these could have served as reservoirs for \textit{S. mutans} and contributed to the transmission of \textit{S. mutans} to other sites. However, in the two subjects with no detectable \textit{S. mutans} colonies after the chlorhexidine treatment, a posterior-anterior transmission of \textit{S. mutans} still occurred, indicating that \textit{S. mutans} could have persisted in retention sites which were hardly or not at all affected by the antimicrobial agent and hence survived. Such places could be occlusal fissures, enamel cracks, incipient lesions, or microspaces between dental restorations and cavity walls from which \textit{S. mutans} could slowly recolonize the tooth surfaces. It may also partly explain why a more long-lasting effect was obtained in the anterior region, with its lower number of retention sites. However, it is still possible that the re-appearance of \textit{S. mutans} could be due to a new infection coming from an external source — a colonization which could have started on the posterior teeth as well and from there spread in an anterior direction to other tooth surfaces. This possibility is interesting, because if the re-emergence is due to regrowth, then more efficient ways of disinfecting the teeth, especially the most posterior ones, need to be developed.

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