The antimicrobial effect of a triclosan/copolymer dentifrice on oral microorganisms in vivo

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Clinical studies have demonstrated the associations between dental plaque and the two most prominent chronic and persistent forms of dental disease: caries and periodontal disease.[1] Human dental plaque is composed of large accumulations of bacteria that are found on the teeth, surfaces of the tongue and other oral mucosal surfaces.

MICROBIOLOGICAL STUDIES

The seminal experiments of Löe and colleagues[2] demonstrated the relationship between dental plaque and the development of gingivitis. Early studies examined the role of specific microorganisms within the plaque matrix and their influence on later stages of plaque formation.[3] Microbiological evaluations of plaque biofilm communities obtained from patients at varying stages of dental health demonstrated distinct changes in the number and types of bacteria when subjects progressed from a healthy state to a diseased state.[4-6]

Together, these observations formed the basis of contemporary clinical practice that advocates for plaque control as a method of treating disease. Methods used to

ABSTRACT

Background. The authors compared the in vivo antimicrobial effects on microorganisms from dental plaque, saliva and the tongue in subjects who used a triclosan/copolymer dentifrice and a fluoride dentifrice (control).

Methods. The authors assigned 15 subjects randomly to the control dentifrice or the triclosan/copolymer dentifrice for twice-daily use for one week. They collected samples of plaque, saliva and tongue scrapings six and 12 hours after the final brushing. They analyzed colony-forming units of *Veillonella* species, *Fusobacteria* species, total cultivable anaerobes and hydrogen sulfide (H_2S)–producing bacteria. A one-week washout followed. The authors repeated the protocol with the second dentifrice.

Results. The results showed no differences at baseline. Significant reductions (88 to 96 percent) in oral anaerobic bacteria were observed in the triclosan/copolymer group six and 12 hours after brushing compared with the control group ($P = .001$). *Fusobacteria* decreased by 77 to 92 percent and *Veillonella* decreased by 84 to 89 percent six and 12 hours after brushing in the triclosan/copolymer group versus the control group. The triclosan/copolymer group also demonstrated a significant decrease in H_2S-producing bacteria six and 12 hours after brushing (74 to 85 percent) ($P = .001$).

Conclusions. Brushing with the triclosan/copolymer dentifrice resulted in significant reductions in microorganisms from the three sites compared with the control dentifrice.

Clinical Implications. The triclosan/copolymer dentifrice produced sustained effects on oral bacteria for 12 hours.

Key Words. Oral bacteria; plaque; salivary rinse; tongue; triclosan/copolymer dentifrice; sustained effect.
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control dental plaque include the physical removal of plaque, the interruption of the specific stages in the plaque developmental process[7,8] and patient-directed control of supragingival plaque with dentifrices and mouthrinses formulated with antimicrobial agents.[9] Several clinical studies have demonstrated the inhibitory effects of these antimicrobial dentifrices and mouthrinses on plaque-associated gingivitis.[10-15]

In the mid-1980s, the American Dental Association (ADA) Council on Dental Therapeutics (now the Council on Scientific Affairs) drafted a set of guidelines to be used to assess both the benefits and risks of active agents in formulations designed to reduce plaque and gingivitis.[15] Shortly thereafter, the U.S. Food and Drug Administration (FDA) adopted similar guidelines.[16] Triclosan, 2,4,4´ trichloro-2´-hydroxydiphenyl ether, is a broad-spectrum antibacterial agent that demonstrates in vitro activity against many of the organisms associated with plaque and plaque-associated gingivitis.[17]

A dentifrice containing 0.3 percent triclosan/2 percent copolymer (polyvinyl methyl ether maleic acid) in a 0.243 percent sodium fluoride/silica base has been shown to reduce supragingival plaque in vivo[18] with concomitant reductions in gingivitis. Studies of up to seven months' duration showed that use of this dentifrice resulted in 13 to 59 percent reductions in plaque compared with a placebo dentifrice.[19,20] Recent in vitro studies demonstrated the effect of the triclosan/copolymer formulation in reducing oral malodor and the bacteria associated with oral malodor.[21,22] These in vitro studies, which were based on well-known antimicrobial concepts,[23,24] demonstrated that the triclosan/copolymer dentifrice can have a profound effect on oral bacteria in plaque related to inflammation and oral malodor. No other dentifrice available in the United States has been shown to have a significant effect on plaque and gingivitis in vivo according to six-month studies that followed the ADA guidelines.[15]

Long-term microbiological studies that have been conducted to evaluate the triclosan/copolymer dentifrice have focused on product safety in a clinical setting and, thus, were designed to examine a person's sensitivity on exposure to the product over time.[13,20] As a result of these safety issues, clinical study designs required the participation of many subjects to assess variations among people.

We designed this study initially as a pilot study to provide data to be used in the calculation of statistical power required to determine the number of subjects needed to demonstrate a meaningful reduction in key members of the oral microflora while using an antimicrobial dentifrice. We developed this study to contrast the results from brushing with a triclosan/copolymer dentifrice with those from brushing with a nonantimicrobial control dentifrice. It is desirable to demonstrate that the effect of formulations can be sustained for 12 hours, the typical time between morning and evening toothbrushing.

We evaluated the antimicrobial effects six and 12 hours after brushing with the triclosan/copolymer dentifrice and compared them with those of a commercially available fluoride dentifrice. We assessed both the magnitude and duration of the in vivo antimicrobial activity of each dentifrice against a group of oral bacteria that are prominent members of the oral flora.

SUBJECTS, MATERIALS AND METHODS

The institutional review board of the University of Medicine and Dentistry of New Jersey, Newark, approved the clinical study protocol. The dental examiner (K.M.) screened study subjects and explained fully the nature of the clinical trial to determine their ability to comply with study procedures. We scheduled for a full evaluation those subjects who expressed an interest and completed and signed an informed consent form. Male and female subjects (age range, 18 to 65 years) who had a minimum of 20 natural teeth with facial and lingual scorable surfaces and no significant oral soft-tissue pathology (with the exception of gingivitis) were included in the study. We excluded subjects who had grossly carious teeth, fully crowned teeth or teeth with extensive restorations on the facial and/or lingual surfaces; we also excluded orthodontically banded teeth, abutments and third molars.

Other exclusion criteria included diabetes, hepatic or renal disease, other serious medical conditions, transmittable diseases and conditions such as heart disease. In addition, we excluded subjects with AIDS, rheumatic fever, a heart murmur or mitral valve prolapse, as well as those requiring prophylactic antibiotic coverage before invasive dental procedures. In addition, we excluded subjects who were receiving prescription medications (antibiotics, anti-inflammatory drugs or anticoagulants), those reporting that they were

pregnant or lactating, and those with a history of adverse effects following use of oral hygiene or other personal care products (such as toothpastes). Finally, we excluded from the study people who reported that they had participated in other clinical studies within the last 30 days, including dental plaque/gingivitis clinical studies involving oral care products.

We enrolled 15 volunteers who met the inclusion criteria. They were recruited by word of mouth. All subjects selected for participation completed the study.

For the duration of the study, we monitored subjects' compliance in the following manner. Subjects were required to maintain a written log documenting every time they brushed their teeth with the test materials provided. In addition, we monitored compliance via visual inspection of the toothpaste during the study. We cautioned subjects to refrain from using any other mouthrinses, dentifrices or oral hygiene devices and to record any protocol mishaps. Participation in the study lasted four to five weeks. At the conclusion of the study, all study participants underwent an oral examination.

CLINICAL PROCEDURES

We gave subjects a soft-headed toothbrush and a commercially available fluoride dentifrice (Colgate Winterfresh Gel, Colgate-Palmolive, New York City) to use for one week before undergoing baseline sampling. After this washout phase, subjects reported to the clinical site having refrained from toothbrushing or other oral hygiene practices for 12 hours. The dental examiner (K.M.) collected supragingival plaque from the buccal surfaces of the upper right quadrant (teeth nos. 2 through 8) using a sterile scaler, pooled it and placed it into a tube containing 1 milliliter of sterile phosphate-buffered saline (PBS). He then removed plaque in the same manner from the lower right quadrant (teeth nos. 25 through 31) and placed it into another tube containing 1 mL of PBS.

The dentist collected bacteria from the left and right halves of the dorsum of the tongue by placing a cotton swab at the midline and rolling it toward the lateral border four times (posterior to anterior). He took the swabs from the two halves and suspended them separately in 1 mL of PBS. He gave subjects 10 mL of commercially available potable water that had been filtered through a 0.2-micrometer filter and treated with ultraviolet light. He instructed subjects to rinse with the water for 10 seconds and to expectorate into a 50-mL tube for collection of the saliva-containing oral rinse sample for microbial analysis. These plaque, tongue and salivary rinse samples constituted the baseline samples for microbiological analysis, which we performed for each subject.

Six-hour samples. We assigned subjects randomly to their test dentifrices (a commercially available sodium fluoride dentifrice [control] or a toothpaste containing triclosan/copolymer [Colgate Total, Colgate-Palmolive]). We then instructed subjects to brush twice daily with the assigned toothpaste for seven days. On the seventh day (at 9 a.m.), we instructed subjects to brush with the assigned dentifrice in the clinical laboratory. They then returned to the laboratory at approximately 3 p.m. the same day. The dentist removed plaque from the buccal surfaces of the upper left quadrant (teeth nos. 9 through 15), pooled it and placed it into a tube containing 1 mL of PBS for microbiological analysis. He collected tongue samples from the left half of the tongue for microbial analysis, as described above. The dentist gave subjects 10 mL of distilled water for microbial analysis of the salivary rinse sample. These samples constituted the six-hour samples.

Twelve-hour samples. We instructed subjects to brush that evening at 9 p.m., refrain from brushing the next morning and report to the clinical laboratory at 9 a.m. that day (day 8), or 12 hours after they last brushed. The dentist collected plaque from buccal surfaces of the lower left quadrant (teeth nos. 18 through 24), pooled it and placed it into a tube containing 1 mL of PBS for microbiological analysis. He collected tongue samples from the right half of the tongue. As described above, he also collected salivary rinse samples. These constituted the 12-hour samples.

Second assigned dentifrice. There was a minimum seven-day washout phase between use of the test products. We gave subjects a soft-headed toothbrush and a commercially available nonantimicrobial fluoride dentifrice to use during the washout period. We then gave subjects their second assigned dentifrice (that is, the triclosan/copolymer dentifrice or control dentifrice) and repeated the entire procedure, including baseline sampling, except that we performed collections on the opposite side of the mouth.

Subjects brushed for seven days with the assigned dentifrice and reported to the laboratory

six and 12 hours after the last brushing, as described above. We collected plaque, tongue and salivary rinse samples to complete the two-way crossover design in which the subject acted as his or her own control. One of us (K.M.) did all of the collections and evaluations in a blinded manner.

MICROBIOLOGICAL PROTOCOL

The microbiologist (D.H.F.) subjected all samples to 30 seconds of pulsed (discontinuous) sonic oscillation (Branson 200 Sonifier, Branson Ultrasonics, Danbury, Conn., with a cup horn attachment) (output = 4, duty cycle = 50 percent). Afterward, we serially diluted (10-fold) the samples in PBS to 10^{-4}. To determine total cell counts, we plated aliquots in duplicate on enriched trypticase soy agar (ETSA) for total anaerobic bacteria and selective agars as follows: crystal violet erythromycin (CVE) agar for isolation of *Fusobacteria* species; Veillonella agar for isolation of *Veillonella* species; and oral organisms producing sulfide (OOPS) agar, which contains 5 percent sheep's red blood cells, for enumeration of hydrogen sulfide (H_2S)–producing bacteria (some of which include *Fusobacteria* species, *Veillonella* species, *Peptostreptococci* species, *Campylobacter* species, *Prevotella* species, *Selenomas* species).[13,20-22]

The microbiologist placed dilutions of 1 in 100 to 1 in 100,000 on the agars described above and plated them according to the manufacturer's directions (Spiral Systems Autoplate 4000 Spiral plater, Spiral Biotech, Norwood, Mass.). We incubated the ETSA, CVE, Veillonella and OOPS agar plates using continuous anaerobic techniques at 37 C for five to seven days. We calculated the colony-forming units (CFUs) from dilutions yielding at least 20 colonies per plate. In all cases, the microbiologist had no knowledge of the subject's dentifrice assignment when plating and counting microorganisms.[21,22]

DATA ANALYSIS

We recorded the microorganisms recovered from each of the oral sites after use of both test products at the baseline, six-hour and 12-hour collections. We evaluated plaque, tongue and salivary rinse samples for oral anaerobes, *Fusobacteria* species, H_2S-producing bacteria and *Veillonella* species, quantifying the number of organisms from each oral site as CFU/mL. We compared the effects of the two treatments to determine the percentage differences in organisms between these two groups. We evaluated the bacteria from all three oral sites sampled.

We performed a statistical analysis between groups using Student t test and log-transformed CFU/mL of each bacterial group from the three sites at the six- and 12-hour posttreatment times. Additional t-test analyses compared the baseline samples after the washout phase for all bacteria assessed from the three sites. We reported results as significant if differences reached the $P < .05$ level.

RESULTS

Fifteen subjects participated in the study. All subjects happened to be nonsmokers. Their ages ranged from 27 to 49 years, with a mean age of 40 years. Thirteen subjects (87 percent) were female. Although we showed no preference for either sex, there was a disproportionate representation of women after screening. Because we designed this to be a crossover study, this disproportionate representation is not problematic.[25] There was an equal distribution of African-American and white subjects in the study.

Figures 1 through 4 (pages 1410 and 1411) show the effects of the two dentifrice formulations on each of the four bacterial groups studied six and 12 hours after brushing. In all four figures, baseline samples demonstrated no statistical differences in the numbers of bacteria from the plaque, tongue and saliva when the control dentifrice was compared with the triclosan/copolymer dentifrice.
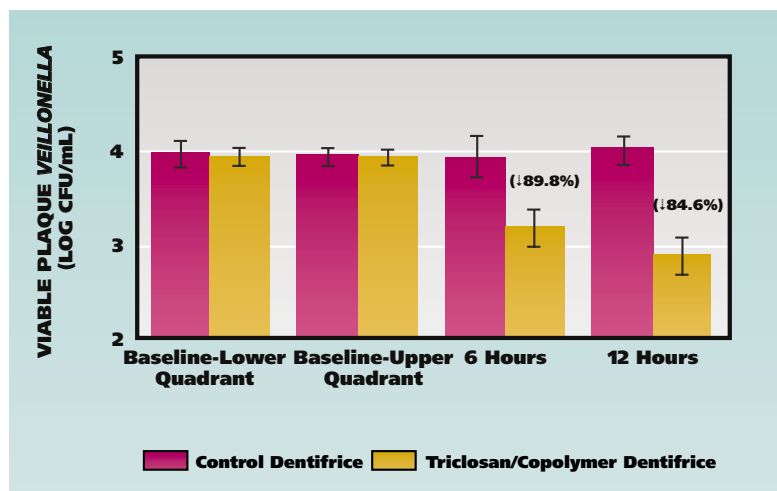
Figure 1 shows that brushing with the triclosan/copolymer dentifrice resulted in statistically significant reductions of 90 percent or better when compared with the control dentifrice for the plaque and tongue anaerobic microflora at both the six- and 12-hour assessments ($P < .05$). In addition, subjects who brushed with the triclosan/copolymer dentifrice exhibited an 88 to 89 percent reduction in salivary anaerobic bacteria compared with those who brushed with the control dentifrice ($P < .05$; data not shown).

Figures 2 through 4 show the results for the *Veillonella* species, *Fusobacteria* species and H_2S-producing oral bacteria, as seen on OOPS agar. At both the six- and 12-hour brushing evaluations, we found a significant decrease in each of the bacterial groups in subjects who used the triclosan/copolymer dentifrice versus the control dentifrice ($P < .05$). The percentage reductions in plaque at the six-hour evaluation were 89.8 percent for *Veillonella* species (Figure 2), 91.2 per-
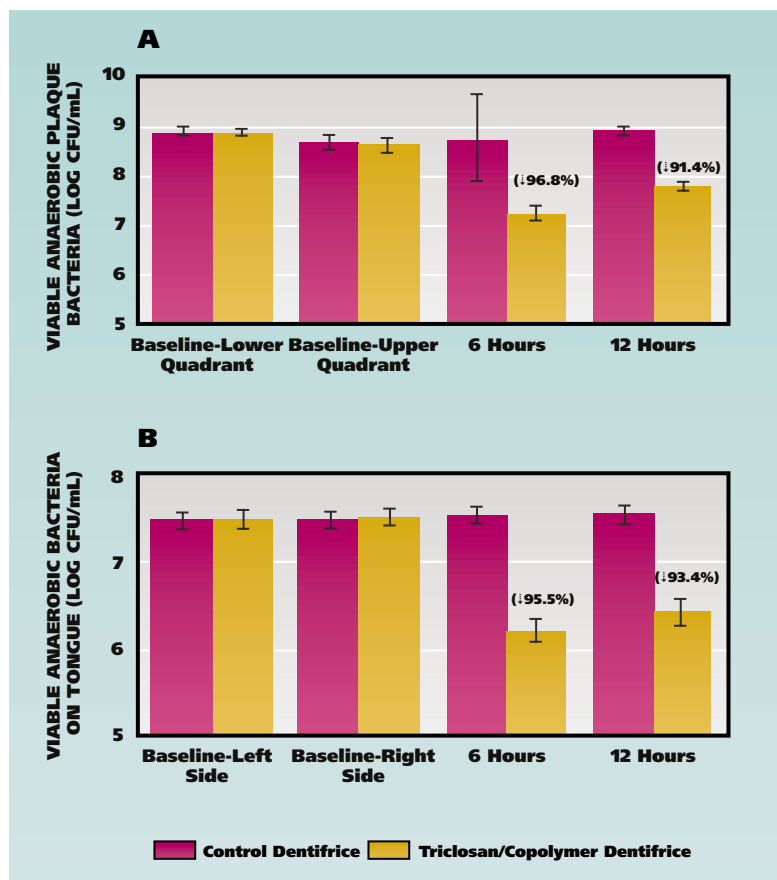
Figure 1. The effects of brushing with the control and triclosan/copolymer dentifrices on anaerobic bacteria from the dental plaque (**A**) and tongue (**B**) six and 12 hours after the last brushing. The bars show the mean viable bacteria (log colony-forming units/milliliter [± standard deviation]) recovered at each sampling point. Statistically significant (P < .05) percentage reductions for the triclosan/copolymer group compared with the control group are shown in parentheses.

Figure 2. The effects of brushing with the control and triclosan/copolymer dentifrices on *Veillonella* species from the dental plaque six and 12 hours after the last brushing. The bars show the mean viable bacteria (log colony-forming units/milliliter [± standard deviation]) recovered at each sampling point. Statistically significant (P < .05) percentage reductions for the triclosan/copolymer group compared with the control group are shown in parentheses.

cent for *Fusobacteria* species (data not shown) and 84.6 percent for H_2S-producing bacteria (data not shown).

Of interest is the fact that the inhibition observed in all microflora groups at 12 hours was similar, albeit slightly lower when compared with the six-hour samples, when we compared the triclosan/copolymer group with the control dentifrice group. Thus, we found reductions of 84.6 percent (versus 89.8 percent) for *Veillonella* (Figure 2), 77.1 percent (versus 91.2 percent) for *Fusobacteria* and 81.1 percent (versus 84.6 percent) for H_2S-producing bacteria. These results are relevant because there does not appear to be any major diminution in the effectiveness of triclosan/copolymer when we compare the 12-hour results with the six-hour results, although in the case of *Fusobacteria* species, the reduction in plaque at 12 hours was 14.1 percent less than that at the six-hour evaluation (data not shown). The maximum difference seen in the other two sites for any of the other bacterial groups was 2.1 percent (a reduction from 95.5 percent to 93.4 percent for total anaerobes on the tongue at six hours versus 12 hours [Figure 1]).

DISCUSSION

Several extensive studies have been performed to document the safety and efficacy of antibacterial mouthrinses and dentifrices that have an effect on plaque and gingivitis.[26] In the mid-1980s, the ADA Council on Dental Therapeutics supported a major effort to develop guidelines that could be used to study the effect of agents used for gingivitis reduction.[15] With slight modifications, the FDA subsequently adopted these guidelines.[16]

The essential elements of these guidelines were a set of suggestions that could be used to test the effect of dentifrices or mouthrinses on gingivitis. Because plaque is tied intimately to gingivitis, the guidelines included clinical measurement of plaque, as well as of gingivitis. Furthermore, if the proposed action of the agent was antimicrobial, documentation was required regarding its effect on the oral microbial flora.
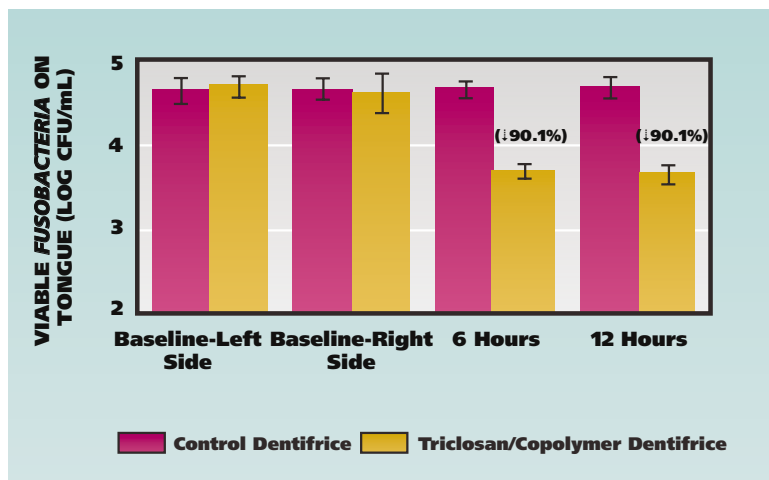
Product safety. Studies that evaluated the efficacy of the triclosan/copolymer dentifrice were designed primarily to evaluate clinical outcomes resulting from the effects of the dentifrice on plaque and gingivitis scores. Microbiological studies included in these evaluations were intended to analyze product safety.[12,13,19] Microbiological outcome measures focused on antimicrobial resistance, potential overgrowth of organisms responsible for caries and periodontal disease, the overgrowth of opportunistic oral pathogens and maintenance of the microbial ecological balance.[15]

In this design, recommended by the ADA Council on Dental Therapeutics[15] and supported by the FDA[16] for the analysis of safety, the broadest range of individual or person-to-person variation is desirable, so that it is possible to determine if any study participant demonstrated any untoward effect with respect to the test agent. Thus, if the active agent produced an undesirable shift in the flora, researchers could analyze it on a person-to-person basis. However, a study design that favors person-to-person variation tends to be biased against group or statistical analysis, because the overall magnitude of the antimicrobial effect varies so widely from person to person that the average effect produced by the agent in question is weakened. Thus, researchers designed these studies not to assess the antimicrobial efficacy of the product, but instead to reveal the rare case in which an adverse microbial outcome occurred in relation to the product.[12,13,19] Hence, a large number of subjects were required to conduct these safety-related studies.

Crossover design. It is well-known that the results of microbiological assessments of plaque vary greatly from person to person, both in the quality and quantity of microorganisms.[27] This broad variation severely compromises statistical analyses aimed at comparing parallel groups of users. One way of overcoming these problems in vivo is to test formulations, under conditions of normal use, with the subject serving as his or her own control in a crossover study design.[28] Moore and colleagues[27] demonstrated that plaque microorganisms taken from two teeth in the same person exhibited less variation than plaque

Figure 3. The effects of brushing with the control and triclosan/copolymer dentifrices on *Fusobacteria* species from the tongue six and 12 hours after the last brushing. The bars show the mean viable bacteria (log colony-forming units/milliliter [± standard deviation]) recovered at each sampling point. Statistically significant ($P < .05$) percentage reductions for the triclosan/copolymer group compared with the control group are shown in parentheses.
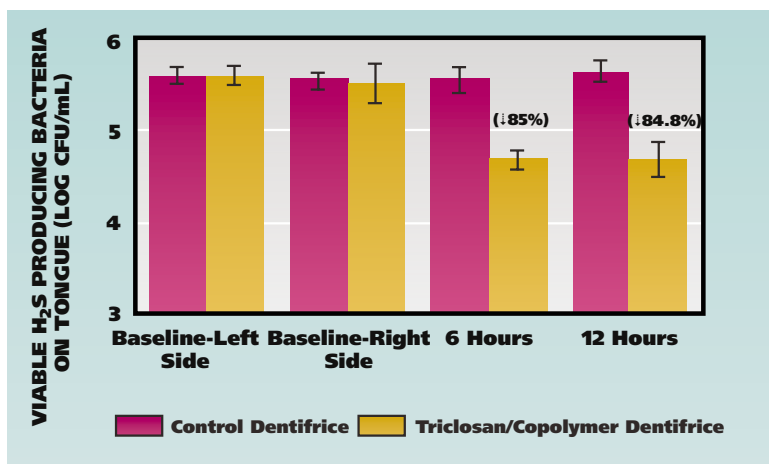
Figure 4. The effects of brushing with the control and triclosan/copolymer dentifrices on oral hydrogen sulfide (H_2S)–producing bacteria from the tongue six and 12 hours after brushing. The bars show the mean viable bacteria (log colony-forming units/milliliter [± standard deviation]) recovered at each sampling point. Statistically significant ($P < .05$) percentage reductions for the triclosan/copolymer group compared with the control group are shown in parentheses.

microorganisms taken from the same tooth in two people. Use of a crossover design enabled our group of researchers to obtain valuable data from relatively small numbers of subjects in studies that evaluated bacterial back spray, the aerosol generated in procedures such as ultrasonic scaling, and bacteremia, resulting from dental scaling.[29,30]

Fine and colleagues[31] used this model to demonstrate the antimicrobial efficacy of an oral care product that had been shown to reduce clinical levels of plaque in large multicenter studies.

The crossover design was useful in demonstrating the antimicrobial basis for the plaque reduction seen in these large clinical trials. Initially, we designed the present study to calculate sample size. We included a six-hour collection period to help determine the biological efficacy of the dentifrice, and we assumed that the optimal benefit would be seen if a reduction was sustained for a 12-hour period.

Because the study showed statistically significant reductions at both the six-hour and 12-hour collection periods in all three sites studied (plaque, saliva and tongue) and in all bacterial groupings (*Veillonella* species, *Fusobacteria* species, total anaerobic bacteria and H_2S-producing bacteria), we are satisfied that this investigation provided convincing data to support the sustained antimicrobial benefits of triclosan, as formulated in the dentifrice tested. In contrast, other dentifrices that routinely contain sodium lauryl sulfate, such as the control dentifrice used in this study, have minimal antimicrobial activity that is short-lived.

Our evaluation included microorganisms collected from three major oral sources: the tongue, plaque and saliva. Furthermore, we chose to examine all oral anaerobes, H_2S-producing bacteria, *Fusobacteria* species and *Veillonella* species. We chose *Fusobacteria* and *Veillonella* because they represent microorganisms that predominate in plaque found in healthy patients and in patients with gingivitis and periodontitis.[4,5] In addition, these organisms are found routinely in supragingival and subgingival plaque. H_2S-producing bacteria are associated with oral malodor.[32,33] An evaluation of oral anaerobes produces a reasonable estimate of the total cultivable microflora.

The results of this study demonstrated that the anaerobes from all three sites were reduced by 90 percent from baseline in subjects who used the triclosan/copolymer dentifrice. We also observed this dramatic reduction in tongue and salivary flora in the triclosan/copolymer group when we assessed *Fusobacteria*. In all other cases, we found reductions of 80 percent or more (with the exception of H_2S-producing organisms on the tongue).

Additional analyses compared the differences at the six- and 12-hour evaluations between samples collected from subjects using the triclosan/copolymer or control dentifrices. Compared with the control dentifrice, the triclosan/copolymer dentifrice resulted in significantly lower numbers of bacteria from the three oral sites evaluated. The percentage reductions in oral anaerobic bacteria and *Vellionella* species ranged from 84 to 96.8 percent. For *Fusobacteria* species and H_2S-producing bacteria, a comparison between the control and triclosan/copolymer formulations demonstrated reductions from 80 to 90 percent for most of the samples assessed. Furthermore, in all instances, the percentage reductions in the triclosan/copolymer group were more than 75 percent in comparison with the control dentifrice group. In addition, the significant effects observed in the triclosan/copolymer group at six hours were maintained at the 12-hour analysis.

Thus, when we compared the triclosan/copolymer group with the control dentifrice group at both the six- and 12-hour collection periods, we found a reduction of approximately one log, or a 10-fold reduction, in all three oral sites for all bacterial groups assessed. These results attest to the oral antimicrobial activity of this triclosan-containing dentifrice under conditions of normal use.

We should point out that the 12-hour period assessed in this study was from 9 p.m. to 9 a.m., which implies that at least six of these hours were during sleep. Dawes and Ong[34] showed that salivary flow varies during the day and is reduced during sleep. As a result, one would expect an increase in bacterial growth potential during sleep. Similarly, a dentifrice with antimicrobial activity that is used before sleep also would be retained longer in the mouth compared with daytime use, because of the reduction in salivary flow. While it seems logical that the antimicrobial effect of a dentifrice used just before sleep would counterbalance the increased potential for bacterial growth overnight, the effect still is not understood fully. In any case, our comparison of two groups that were treated equally supports the antibacterial effect seen in this study. Moreover, data suggesting that this diurnal effect will vary from person to person lend credence to the use of a subject as his or her own control, as we did in this crossover study.[35]

CONCLUSION

We designed this study to investigate the effect of a triclosan/copolymer dentifrice on the relative distribution of microorganisms in three oral sites (plaque, saliva and the tongue) six hours and 12 hours after use. Our results demonstrate that

twice-daily use of the triclosan/copolymer dentifrice for one week produced a bacterial reduction approaching one log in each of the bacterial parameters at each oral site assessed. Furthermore, the results demonstrate that this effect was sustained for up to 12 hours after the last application of the dentifrice, and they represent a statistically significant antimicrobial effect. These results also are consistent with observations from a large number of clinical studies that demonstrated beneficial reductions in dental plaque with the use of a triclosan/copolymer dentifrice.[14,18-20] ∎

1. Löe H. Research on oral diseases and its impact on dental education and practice. Adv Dent Res 1988;2(2):199-203.

2. Löe H, Theilade E, Jensen SB, Schiott CR. Experimental gingivitis in man, part III: the influence of antibiotics on gingival plaque development. J Periodont Res 1967;2(4):282-9.

3. Moore WE, Moore LH, Ranney RR, Smibert RM, Burmeister JA, Schenkein HA. The microflora of periodontal sites showing active destructive progression. J Clin Periodontol 1991;18(10):729-39.

4. Moore LV, Moore WE, Cato EP, et al. Bacteriology of human gingivitis. J Dent Res 1987;66(5):989-95.

5. Socransky SS, Haffajee AD, Smith C, Dibart S. Relation of counts of microbial species to clinical status at the sampled site. J Clin Periodontol 1991;18(10):766-75.

6. Ximenez-Fyvie LA, Haffajee AD, Socransky SS. Comparison of the microbiota of supra- and subgingival plaque in health and periodontitis. J Clin Periodontol 2000;27(9):648-57.

7. Smulow JB, Turesky SS, Hill RG. The effect of supragingival plaque removal on anaerobic bacteria in deep periodontal pockets. JADA 1983;107(5):737-42.

8. Kho P, Smales FC, Hardie JM. The effect of supragingival plaque control on the subgingival microflora. J Clin Periodontol 1985;12(8):676-86.

9. Van Strydonck DA, Demoor P, Timmerman MF, van der Velden U, van der Weijden GA. The anti-plaque efficacy of a chlorhexidine mouthrinse used in combination with toothbrushing with dentifrice. J Clin Periodontol 2004;31(8):691-5.

10. Gordon J, Lamster I, Seiger M. Efficacy of Listerine antiseptic in inhibiting the development of plaque and gingivitis. J Clin Periodontol 1985;12(8):697-704.

11. Grossman E, Reiter G, Sturzenberger OP, et al. Six-month study of the effects of chlorhexidine mouthrinse on gingivitis in adults. J Periodont Res 1986;21(supplement 16):33-8.

12. Walker C, Borden LC, Zambon JJ, Bonta CY DeVizio W, Volpe AR. The effects of a 0.3% triclosan-containing dentifrice on the microbial composition of supragingival plaque. J Clin Periodontol 1994;21(5):334-41.

13. Zambon JJ, Reynolds HS, Dunford RG, et al. Microbial alterations in supragingival dental plaque in response to a triclosan-containing dentifrice. Oral Microbiol Immunol 1995;10(4):247-55.

14. Mankodi S, Walker C, Conforti N, DeVizio W, McCool JJ, Volpe AR. Clinical effect of a triclosan-containing dentifrice on plaque and ginigivitis: a six-month study. Clin Prev Dent 1992;14(6):4-10.

15. Guidelines for acceptance of chemotherapeutic products for the control of supragingival plaque and gingivitis. Council on Dental Therapeutics. JADA 1986;112(4):529-32.

16. Guidance for industry on development and evaluation of drugs for treatment or prevention of gingivitis. Fed Regist 2003;70(163). Docket no. 2005D-0240.

17. DeSalva SJ, Kong BM, Lin YJ. Triclosan: a safety profile. Am J Dent 1989;2(special number):185-96.

18. Palomo F, Wantland L, Sanchez A, DeVizio W, Carter W, Baines E. The effect of a dentifrice containing triclosan and a copolymer on plaque formation and gingivitis: a 14-week clinical study. Am J Dent 1989;2(special number):231-7.

19. Garcia-Godoy F, Garcia-Godoy F, DeVizio W, Volpe AR, Ferlauto RJ, Miller JM. Effect of a triclosan/copolymer/fluoride dentifrice on plaque formation and gingivitis: a 7-month clinical study. Am J Dent 1990;3(special number):S15-S26.

20. Fine DH, Furgang D, Bonta Y, et al. Efficacy of a triclosan/NaF dentifrice in the control of plaque and gingivitis and concurrent oral microflora monitoring. Am J Dent 1998;11(6):259-70.

21. Furgang D, Sreenivasan PK, Zhang YP, Fine DH, Cummins D. The effects of a new therapeutic triclosan/copolymer/sodium-fluoride dentifrice on oral bacteria, including odorigenic species. Compend Contin Educ Dent 2003;24(supplement 9):14-9.

22. Sreenivasan PK, Furgang D, Zhang Y, DeVizio W, Fine DH. Antimicrobial effects of a new therapeutic liquid dentifrice formulation on oral bacteria including odorigenic species. Clin Oral Invest 2005;9(1):38-45.

23. Edberg SC. In vitro antimicrobial susceptibility testing. In: Edberg SC, Berger SA, eds. Antibiotics and infection. New York: Churchill Livingstone; 1983.

24. Sherris JC. Antimicrobials and chemotherapy. In: Sherris JC, Ryan KJ, eds. Medical microbiology: An introduction to infectious diseases. New York: Elsevier; 1984.

25. Sibbald B, Roberts C. Understanding controlled trials: crossover trials. BMJ 1998;316(7146):1719.

26. Fine DH. Chemical agents to prevent and regulate plaque development. Periodontol 2000 1995;8:87-107.

27. Moore WE, Holdeman LV, Cato EP, et al. Variation in periodontal floras. Infect Immun 1984;46(3):720-6.

28. Fine DH, Furgang D, Steinberg LM, et al. A model for clinical evaluation of anti-microbial effects of agents on plaque colonization. Am J Dent 2000;13(3):153-8.

29. Fine DH, Yip J, Furgang D, Barnett ML, Olshan AM, Vincent J. Reducing bacteria in dental aerosols: pre-procedural use of an antiseptic mouthrinse. JADA 1993;124(5):56-8.

30. Fine DH, Korik I, Furgang D, et al. Assessing pre-procedural subgingival irrigation and rinsing with an antiseptic mouthrinse to reduce bacteremia. JADA 1996;127(5):641-6.

31. Fine DH, Furgang D, Sinatra K, Charles C, McGuire A, Kumar LD. In vivo antimicrobial effectiveness of an essential oil-containing mouth rinse 12 h after a single use and 14 days' use. J Clin Periodontol 2005;32(4):335-40.

32. Minah GE, Turng B-F. Microbiological and ecological aspects of oral malodor. Res Adv Microbiol 2000;1:43-59.

33. Paryavi-Gholami F, Minah GE, Turng BF. Oral malodor in children and volatile sulfur compound-producing bacteria in saliva: preliminary microbiological investigation. Pediatr Dent 1999;21(6):320-4.

34. Dawes C, Ong BY. Circadian rhythms in the flow rate and proportional contribution of parotid to whole saliva volume in man. Arch Oral Biol 1973;18(9):1145-53.

35. Ferguson DB, Fort A. Circadian variations in human resting submandibular saliva flow rate and composition. Arch Oral Biol 1974;19(1):47-55.