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The antimicrobial efficacy of commercial dentifrices

Violet I. Haraszthy, DDS, MS, PhD ▪ Joseph J. Zambon, DDS, PhD ▪ Prem K. Sreenivasan, PhD

This investigation compared the effects of a fluoride dentifrice and toothpastes formulated with antimicrobial ingredients (stannous fluoride and triclosan/copolymer) on oral micro-organisms, including those found in samples taken from the human oral cavity. Microbiological techniques determined the minimum inhibitory concentrations (MICs) of each dentifrice necessary to inhibit the growth of bacterial strains from the healthy oral cavity, as well as those found in dental caries, periodontal disease, and halitosis. *Ex vivo* studies utilized oral rinse samples and supragingival plaque from adults to determine antimicrobial effects on the entire microbial diversity of these samples, including biofilm-derived micro-organisms.

The triclosan/copolymer dentifrice demonstrated the lowest MICs and significantly inhibited Gram-positive and Gram-negative bacteria (including the periodontal pathogens *Aggregatibacter actinomycetemcomitans*, *Eikenella corrodens*, and *Fusobacterium nucleatum*). In the *ex vivo* tests, the triclosan/copolymer dentifrice demonstrated substantial inhibition in the oral rinse samples over each treatment period ($p > 0.0005$) as compared to either the fluoride or stannous fluoride dentifrices. Similarly, the triclosan/copolymer dentifrice demonstrated the highest inhibition of micro-organisms in the supragingival plaque biofilm ($p < 0.0005$). No significant differences were observed between the fluoride and stannous fluoride dentifrices ($p > 0.5$).

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The human oral cavity is home to large densities of endogenous micro-organisms, including a variety of Gram-positive and Gram-negative bacteria.[1] The mouth's warm and moist environment, together with its unique anatomical features (such as non-shedding surfaces and soft tissues) and the nutrients it contains, provides a range of factors optimal for microbial proliferation. Several microbiological investigations have focused on defining the characteristics of these oral micro-organisms.[2-4] Collectively, these studies have utilized a variety of microbiological techniques to characterize the micro-organisms in saliva and in the natural biofilms attached to the surfaces of exposed teeth and soft tissues (including the tongue and cheeks). Together, these studies highlight several groups of micro-organisms that are prevalent in oral disease.[1]

One of the most extensively studied natural biofilms in health and oral disease is supragingival dental plaque.[1] Clinical studies demonstrate that unrestricted accumulations of dental plaque are associated with the initiation and progression of oral diseases, including gingivitis, caries, and periodontal disease.[5,6] Effective oral hygiene plays an important role in reducing dental plaque and maintaining oral health; however, despite educational programs to improve oral hygiene, it is clear that most individuals do not or cannot practice optimal oral hygiene.[7,8] Studies indicate that approximately 66% of all individuals who claimed to brush their teeth twice a day had deposits of dental plaque on their teeth.[9,10] Additionally, the inability to optimally clean tooth surfaces is reflected in the presence of dental plaque in one-third of all teeth immediately after brushing.[8] Knowing this, the global prevalence of oral diseases (such as gingivitis) is not surprising; most populations report severe periodontitis in 5–10% of adults and gingivitis in the majority of children and adolescents.[6]

A number of approaches for improving routine oral hygiene have been documented in the literature.[11] One common approach is the routine use of dentifrices formulated with antimicrobial ingredients as an important adjunct to help control the dental plaque biofilm.[12-14] Antimicrobial ingredients that provide the most benefit are those that offer broad-spectrum activity on oral micro-organisms, are safe for routine use, and improve oral health parameters.[14]

The current study examined the effects of commercial dentifrices formulated with stannous fluoride, triclosan/copolymer, and fluoride on the micro-organisms commonly found in the human oral cavity. The minimum inhibitory concentration (MIC) for each dentifrice—that is, the lowest concentration of dentifrice required to inhibit the growth of each individual micro-organism, including oral bacteria, periodontal pathogens, oral yeasts, and non-oral bacteria—is listed in the table. *Ex vivo* tests determined the antimicrobial effects of each dentifrice on the cultivable micro-organisms found in these samples.

This *ex vivo* test was designed to incorporate the inherent microbiological variations between subjects and include micro-organisms derived from natural habitats. Compared to cultures grown in the laboratory, natural populations include clinical strains of micro-organisms that proliferate under diverging environmental conditions of pH, nutrition, and oxygen tension.[1,15,16] Oral samples were collected from adult subjects to determine the effects of different treatment durations on microbial viability. The second type of *ex vivo* test determined the effects of the dentifrices on the supragingival plaque biofilms from adults.

Table. MIC (µg/mL)of each dentifrice required to inhibit the growth of test organisms.

Bacterial species	Strain number	Dentifrice: Fluoride toothpaste	Dentifrice: Crest Pro-Health	Dentifrice: Colgate Total
Actinomyces meyeri	ATCC 33972	15	75	15
Actinomyces viscosus	ATCC 43146	7.5	7.5	7.5
Aggregatibacter actinomycetemcomitans	ATCC 43717	3.5	3.5	<0.94
Aggregatibacter actinomycetemcomitans Y4	ATCC 43718	30	3.5	1.8
Bacillus cereus	ATCC 11778	15	15	7.5
Bacillus subtilis	ATCC 6051	>150	>150	15
Campylobacter rectus	ATCC 33238	3.5	7.5	1.8
Candida albicans	ATCC 90028	150	150	30
Capnocytophaga gingivalis	ATCC 33124	3.5	75	3.5
Escherichia coli	ATCC 4157	150	150	7.5
Eikenella corrodens	ATCC 23834	15	15	<0.94
Fusobacterium nucleatum	ATCC 25586	7.5	7.5	1.8
Moraxella catarrhalis	ATCC 8176	1.8	3.5	<0.94
Porphyromonas gingivalis	ATCC 53977	1.8	1.8	1.8
Prevotella intermedia	ATCC 25611	3.5	3.5	3.5
Prevotella melaninogenica	ATCC 25845	3.5	3.5	3.5
Prevotella nigrescens	NCTC 9336	1.8	1.8	1.8
Solobacterium moorei	J10654	30	30	30
Staphylococcus aureus	ATCC 6538	75	30	15
Streptococcus gordonii	ATCC 10558	7.5	7.5	3.5
Streptococcus mutans	ATCC 6538	7.5	7.5	7.5
Veillonella dispar	ATCC 17748	15	30	15
Veillonella atypica	ATCC 27215	7.5	7.5	3.5

Materials and methods

Micro-organisms

The micro-organisms used for this investigation were obtained from the culture collection at the University of Buffalo School of Dental Medicine or the American Type Culture Collection in Manassas, Virginia. The study utilized 19 oral and 4 non-oral strains that were maintained with enriched tryptic soy agar supplemented with 5% defibrinated sheep blood, 5.0 µg/mL hemin, and 0.5 µg/mL vitamin K_1.

Dentifrices

Test dentifrices were obtained commercially and included a generic 0.243% sodium fluoride (NaF) toothpaste, a dentifrice formulated with stannous fluoride (SnF_2) (Crest Pro-Health, Procter & Gamble Co.), and a triclosan/copolymer toothpaste (Colgate Total, Colgate-Palmolive). Slurries of all dentifrices were prepared in sterile water prior

to the studies and further diluted as required for each test.

MIC test

This test examined the smallest amount of dentifrice slurry capable of inhibiting the growth of micro-organisms. Bacteria for these studies were routinely grown in broth media and diluted prior to the test. Dilutions of dentifrice slurries were incubated with each bacterial culture. The smallest concentration of each dentifrice to inhibit bacterial growth was recorded. Bacterial samples with no added treatment were included as controls.

Ex vivo *antimicrobial tests*

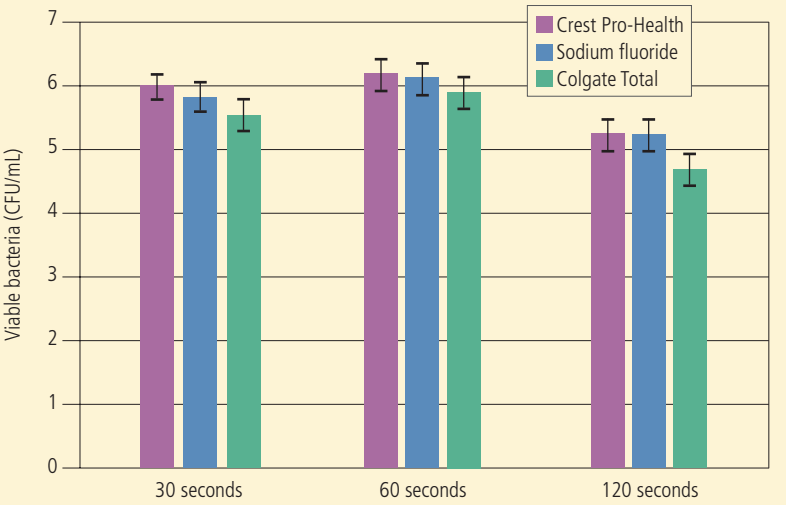
Human volunteers and oral sample collection

Eighteen adults aged 18–70 completed and signed an informed consent. Subjects were provided with a commercially available fluoride dentifrice to use for one week prior to the start of the study. On the day of the study, the subjects refrained from oral hygiene prior to arriving at the dental clinic. Oral samples were collected as described previously.[17,18]

To determine the antimicrobial effects of dentifrices on oral rinse samples collected from adults, subjects rinsed with 10 mL of sterile water for 10 seconds. These rinse samples were collected in sterile tubes, and aliquots of these samples were treated with each dentifrice slurry for 30, 60, and 120 seconds. Oral samples were treated as described previously and diluted in buffer prior to plating dilutions on defibrinated sheep blood. Agar media were incubated under anaerobic conditions at 37°C for five days to quantify viable bacteria.

Ten adults participated in an additional *ex vivo* study that examined the effects of the three dentifrices on supragingival plaque. The effects were tested in accordance with procedures described previously.[18] Supragingival plaque was collected from the 10 adult subjects after informed consent. Plaque samples were collected by a dentist and transported immediately to the laboratory. Samples from the subjects were sonicated briefly and dilutions of these samples were distributed onto media prepared with different concentration of each test dentifrice. For a control, plaque samples were also distributed on untreated media. All media were incubated at 37°C. The numbers of viable bacteria (CFU/mL) were recorded from all media for each subject.

Chart 1. The antimicrobial effects of test dentifrices on oral bacteria.



Statistical analyses

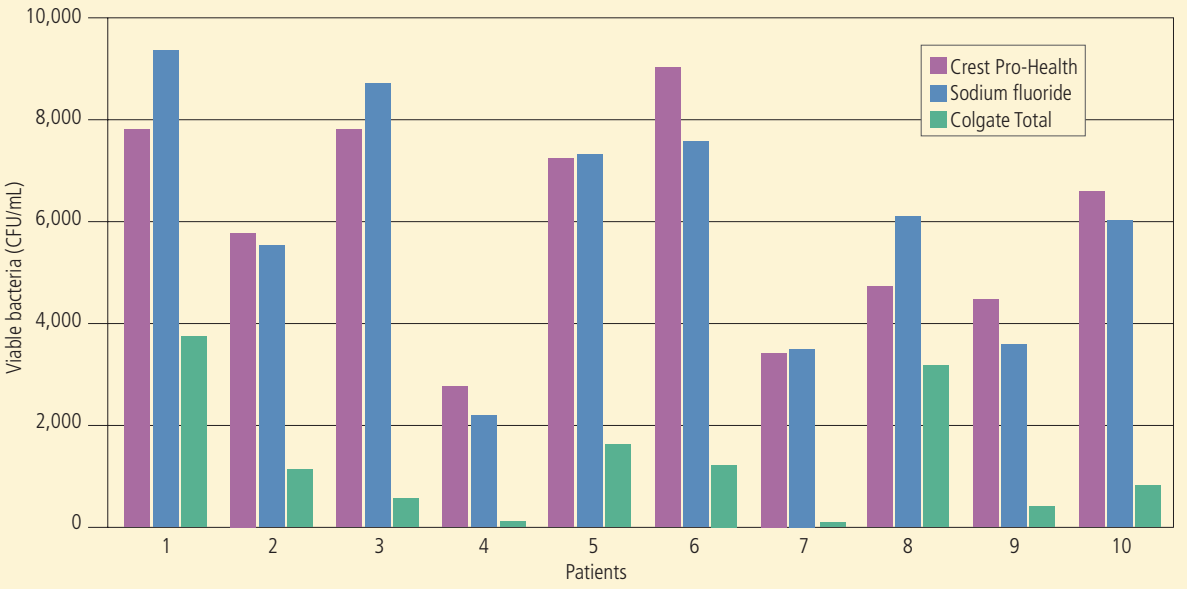
Results from the *ex vivo* tests were analyzed by ANOVA (95% confidence level). Significant results were further analyzed by *post-hoc* multiple comparison Tukey's tests with subjects and dentifrice in the model. Analyses were conducted by Minitab (Minitab Inc.) and results at $p < 0.05$ were reported as significant.

Results

The MICs of the three dentifrices are shown in the table. Colgate Total demonstrated significantly higher activity than either Crest Pro-Health or the sodium fluoride toothpaste ($p < 0.05$). Colgate Total's MICs for oral bacteria started at less than 0.94 µg/mL and went as high as 30 µg/mL. By comparison, MICs were higher for Crest Pro-Health, ranging from 1.8 to more than 150 µg/mL. The differences in the MICs between Colgate Total and Crest Pro-Health were four-fold for Gram-negative micro-organisms such as *Aggregatibacter actinomycetemcomitans, Campylobacter rectus, Eikenella corrodens,* and *Fusobacterium nucleatum*. For a majority of the bacteria (including both Gram-positive and Gram-negative micro-organisms), similar MICs were observed for sodium fluoride and Crest Pro-Health. Among the dentifrices tested, Crest Pro-Health demonstrated the highest MIC for the oral bacteria *Capnocytophaga gingivalis* and *Actinomyces meyerii.*

Chart 1 shows the results of the

Chart 2. The effects of dentifrices on supragingival plaque bacteria for each patient.

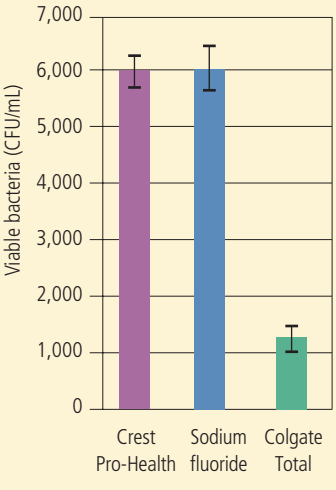


oral rinse samples for the 18 adults treated with dentifrice for 30, 60, and 120 seconds. Results indicate average microbial viability after each treatment. An initial ANOVA on the entire dataset indicated that the dentifrice duration interaction (that is, dentifrice efficacy within each time period) did not differ significantly between dentifrices ($p = 0.297$). A final statistical model involving subject, dentifrice, and duration indicated that the effects of all three were significant ($p < 0.0005$).

Additional analyses were conducted at each of the three treatment durations (that is, 30, 60, and 120 seconds). This two-way ANOVA with subject and dentifrice was used for analysis. At all three treatment durations, subjects and dentifrices were statistically significant ($p < 0.0005$). In multiple comparison tests, Colgate Total was more effective than Crest Pro-Health and sodium fluoride at the 30-second post-treatment assessment ($p < 0.0005$). At 60 seconds post-treatment, Colgate Total was still more effective than Crest Pro-Health ($p < 0.0005$) and sodium fluoride ($p = 0.0001$); in addition, sodium fluoride demonstrated better effects than Crest Pro-Health ($p = 0.029$). At 120 seconds post-treatment, Colgate Total was more effective than Crest Pro-Health ($p < 0.0005$) and sodium fluoride ($p < 0.0005$), although sodium fluoride was not significantly different from Crest Pro Health at this interval ($p = 0.058$).

Chart 2 shows microbial viability for the supragingival plaque samples following treatment with each test dentifrice. A two-way ANOVA with subject and dentifrice as effects was used to analyze the results and demonstrated that the dentifrices produced significant effects ($p < 0.0005$). Colgate Total demonstrated the greatest effects on plaque compared with Crest Pro-Health and sodium fluoride. Chart 3 shows the average microbial viability after treatment with each dentifrice. *Post-hoc* multiple comparison Tukey's tests indicate that Colgate Total demonstrated significantly higher bacterial growth inhi-

Chart 3. The average effect of dentifrices on supragingival bacteria for all patients.



bition than either Crest Pro-Health or sodium fluoride ($p < 0.0005$). No significant differences were observed between sodium fluoride and Crest Pro-Health ($p = 0.99$).

Discussion

Assessing the antimicrobial effects of commercial dentifrices (including those formulated with ingredients designed to control oral bacteria) was a primary focus of this study. Antimicrobial tests have been used elsewhere in the literature to assess the effects of oral hygiene formulations.[11] Many of these procedures utilize isolated strains of oral bacteria to determine the lowest concentration of agents required to inhibit microbial growth. In contrast to previous studies, the present study determined the effects of dentifrices on a battery of common oral bacteria associated with oral health and on micro-organisms found in halitosis, caries, and periodontal disease.[18] Colgate Total, the dentifrice containing triclosan/copolymer, demonstrated lower MICs than the other dentifrices tested and inhibited the entire group of Gram-positive and Gram-negative bacteria. Based on the results of this study, Colgate Total had a substantially greater effect on Gram-negative pathogens (including *Aggregatibacter actinomycetemcomitans, E. corrodens,* and *F. nucleatum*), Gram-positive organisms such as streptococci, oral yeasts such as *Candida albicans,* and other non-oral bacteria, including staphylococci and *Bacillus* spp.

As recent studies have highlighted critical differences between laboratory strains and those isolated from clinical samples, the use of clinical strains in tests for antimicrobial activity constitutes an important variable.[16] Strains of *Veillonella, Porphyromonas gingivalis,* and streptococci isolated from oral samples demonstrate clonal differences between individuals.[19,20] These differences are reflected in variations in antimicrobial susceptibilities and virulence properties.[15]

In addition, the human oral cavity undergoes numerous environmental changes during the course of a day, due to natural physiological processes that influence microbial physiology and virulence traits.[1] While the physiological reasons for these variations remain unclear, the literature has indicated regional differences in the microflora (for example, tongue versus saliva) and the transitory nature of these populations.[21] Other studies have assessed these variations amongst *Streptococcus mutans* and *Actinomyces naeslundii.*[3,22] For the present study, samples of dental plaque and saliva were collected from adult subjects to evaluate the individual differences in the natural populations of oral microflora.

The *ex vivo* tests collected oral samples to include the microbial variations observed in the mouth. The *ex vivo* tests require few preparatory steps for retaining the physiological characteristics of the collected oral samples prior to antimicrobial assessments. Utilizing these samples, Colgate Total demonstrated significantly greater effectiveness on samples collected from all patients than did Crest Pro-Health or sodium fluoride. Significant effects were observed for Colgate Total at each treatment period chosen to reflect time exposures reported for toothbrushing.[8,9] Multiple comparison statistical analyses that included subject and dentifrices in the assessment revealed no differences between the efficacy of Crest Pro-Health and sodium fluoride.

A separate component of the *ex vivo* tests examined the effects of the test dentifrices on the supragingival plaque collected from adults. It is now widely recognized that the micro-organisms in biofilms have significantly different physiology and antimicrobial properties compared to sessile bacteria.[15] For instance, biofilms are less susceptible to antimicrobials and demonstrate considerable physiologic variations within their organized structure.[1] Furthermore, certain types of bacteria are particularly common in supragingival plaque.[20]

This study collected natural biofilms formed on the exposed surfaces of the teeth, utilizing standardized procedures to maintain the microbial variations within each sample. All dental plaque samples were treated with each dentifrice, utilizing identical procedures to maintain these microbial variations within each sample. For each supragingival plaque sample tested, Colgate Total demonstrated significantly higher antimicrobial effects than the other test dentifrices. Statistical analyses demonstrated no differences in antimicrobial effects between Crest Pro-Health and sodium fluoride.

Conclusion

This investigation utilized several different methods to determine the antimicrobial effects of dentifrices. The results demonstrate the significant effects of the triclosan/copolymer dentifrice on both oral bacteria and on the micro-organisms derived from supragingival biofilms. These results are consistent with earlier clinical studies that indicated the clinical efficacy of triclosan/copolymer in reducing dental plaque and gingivitis.[12,13] Corresponding meta-analyses

of stannous fluoride dentifrices indicate that they have an antigingivitis effect but a less prominent effect on dental plaque.[12,23]

Disclaimer

Dr. Sreenivasan is a manager at the Colgate-Palmolive Advanced Technology Center.

Author information

Dr. Haraszthy is an associate professor of Restorative Dentistry, University at Buffalo, School of Dental Medicine in New York, where Dr. Zambon is a SUNY distinguished teaching professor of Periodontics and Oral Biology and associate dean for academic affairs. Dr. Sreenivasan is manager of Clinical Dental Research, Colgate-Palmolive Advanced Technology Center, Piscataway, New Jersey.

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Manufacturers

Colgate-Palmolive, New York, NY
800.763.0246, www.colgate.com

Minitab Inc., State College, PA
800.448.3555, www.minitab.com

Procter & Gamble Co., Cincinnati, OH
800.543.2577, www.pg.com