Clin Oral Invest (2005) 9:38–45
DOI 10.1007/s00784-004-0285-0

ORIGINAL ARTICLE

P. K. Sreenivasan · D. Furgang · Y. Zhang · W. DeVizio · D. H. Fine

Antimicrobial effects of a new therapeutic liquid dentifrice formulation on oral bacteria including odorigenic species.

Received: 16 February 2004 / Accepted: 17 August 2004 / Published online: 22 September 2004

Abstract The control of oral malodor is well-recognized in efforts to improve oral health. Antimicrobial formulations can mitigate oral malodor, however, procedures to assess effects on oral bacteria including those implicated in halitosis are unavailable. This investigation examined the antimicrobial effects of a new liquid triclosan/copolymer dentifrice (test) formulation that demonstrated significant inhibition of oral malodor in previous organoleptic clinical studies. Procedures compared antimicrobial effects of the test and control formulations on a range of oral micro-organisms including members implicated in halitosis, substantive antimicrobial effects of formulations with hydroxyapatite as a surrogate for human teeth and ex vivo effects on oral bacteria from human volunteers. With *Actinomyces viscosus*, as a model system, the test formulation demonstrated a dose-dependent effect. At these concentrations the test formulation provided significant antimicrobial effects on 13 strains of oral bacteria including those implicated in bad breath at selected post-treatment time points. Treatment of hydroxyapatite by the test dentifrice resulted in a significant and substantive antimicrobial effect vs. controls. Oral bacteria from subjects treated ex vivo with the test dentifrice resulted in significant reductions in cultivable oral bacteria and odorigenic bacteria producing hydrogen sulfide. In summary, microbiological methods adapted to study odorigenic bacteria demonstrate the significant antimicrobial effects of the test (triclosan/copolymer) dentifrice with laboratory and clinical strains of oral bacteria implicated in bad breath.

P. K. Sreenivasan · Y. Zhang · W. DeVizio
Colgate-Palmolive Company,
909 River Road, Piscataway, NJ 08855, USA

D. Furgang · D. H. Fine (✉)
Oral Biology Department,
University of Medicine and Dentistry of New Jersey,
MSB, 185 South Orange Avenue, Newark, NJ 07103-2714, USA
e-mail: finedh@umdnj.edu
Tel.: +1-973-9723728
Fax: +1-973-9720045

Keywords Antimicrobial · Bacteria · Malodor · Substantivity · Triclosan

Introduction

Oral malodor due to its antecedent social and psychological handicap has a long history of research and is well recognized in the literature from Greek and Roman times with remedies that can be traced to Hippocrates. In contemporary surveys oral malodor is an important reason for patient visits to dentists [9, 10, 11, 14, 16, 19]. Although epidemiological data on the prevalence of malodor among various populations is unavailable, estimates suggest approximately one-half the North American population and 24% of Japanese individuals complain of malodor [11, 16].

The etiology of oral malodor is complex with origins from the oral cavity reported in a majority of cases and nonoral sources such as physiological or pathological reasons among others [9]. Research suggests a role for microbial metabolism including the putrefaction of food and desquamated epithelial cells among the principal reasons for oral malodor [9, 14, 19]. Copious amounts of volatile sulfur compounds (VSCs) including hydrogen sulfide, methyl mercaptan, dimethyl sulfide, and minor components including amines and acids from diverse substrates are produced by oral micro-organisms associated with malodor [2, 6, 9, 12, 13, 22, 23]. The organoleptic (or hedonic) clinical procedure with trained judges is widely accepted for malodor determinations [9]. In recent years instrumental methods including gas chromatography with different detectors have been described to quantify VSCs with the development of a portable device (Halimeter, Interscan, Chatsworth, Calif., USA) also reported to record oral malodor [9]. Microbiological tests to determine malodor include microbial trypsinase activity (the BANA test) with oral samples [9]. Recent cultural methods selectively quantify oral microbiota producing hydrogen sulfide [10]. Other approaches have utilized molecular biology to identify bacteria in subjects

with halitosis [8] and identify bacterial genes for production of specific VSCs [6, 22, 23].

The objectives of this investigation were to examine the effects of oral care formulations on oral bacteria implicated in malodor utilizing test procedures that were adapted to conditions simulating those found in the human mouth. In vitro methods tested the susceptibilities of a range of oral bacteria including several strains typically found in the mouth in the complex dental biofilms on the tongue and in dental plaque associated with halitosis. Studies also examined the substantivity of dentifrice antimicrobial efficacy with hydroxyapatite as a surrogate for the human tooth [3]. Halitosis-associated bacteria have been reported from several distinct oral niches in the human mouth. Therefore an ex vivo method examined the effects of these dentifrices on the complex mixture of oral bacteria obtained by sampling saliva and tongue scrapings from human volunteers. All investigations were conducted with control dentifrice formulations and a recently described liquid dentifrice formulation with triclosan/copolymer. These results are in line with previous organoleptic clinical trails that demonstrate significant reductions in malodor by the triclosan/copolymer dentifrice [9, 17, 21].

Materials and methods

Bacteriological media/reagents

Dehydrated broth culture media was obtained from Becton-Dickinson (Sparks, Md., USA) and prepared according to manufacturer's instructions. Prepared plates of Trypticase Soy Agar with 5% sheep blood (blood agar) were obtained from Becton-Dickinson. All other agar media were prepared from dehydrated powder (Becton-Dickinson) as needed with the oral hydrogen-sulfide producing organism agar described previously to selectively quantify oral bacteria producing hydrogen sulfide (H_2S) [10]. Phosphate buffered saline (PBS) pH 7.4 was obtained from Life Technologies (Gibco BRL, Grand Island, N.Y., USA). Hydroxyaptite squares (HA) measuring 3×3×1 mm were utilized for some experiments and obtained from ICN Biomedical (Aurora, Ohio, USA). All chemicals were routinely obtained from Sigma Chemical (St. Louis, Mo., USA) unless indicated otherwise.

Bacterial strains

Thirteen different species of oral bacteria (Table 1) were obtained from the American Type Culture Collection (ATCC; Manassas, Va, USA) and routinely cultivated in media as per ATCC recommendations. Typically, laboratory bacterial strains were grown at 37°C in 10 ml of the appropriate liquid media (as shown in Table 1) to early stationary phase and adjusted to A_{560}=0.8 (approx. 10^8 bacteria/ml) for tests.

Dentifrice formulations tested

The three dentifrice formulations tested were: a commercially available fluoride dentifrice (paste A), Colgate Total 2-in-1 liquid (with triclosan/copolymer as the active system and 0.24% sodium fluoride referred to as paste B) and another commercially available fluoride dentifrice containing flavor, humectants, abrasives and sodium fluoride (0.24%) as the active ingredient (paste C). The commercially available pastes (A and C) are formulated with ingredients typical for dentifrices and include flavor, humectants, abrasives, surfactants with sodium fluoride as the active ingredient.

Antimicrobial efficacy of dentifrices with bacterial strains

Initial dose-response effect studies examined the effects of a range of aqueous dentifrice supernatant concentrations on *A. viscosus* as a model system. For these experiments the dentifrice slurries were prepared by stirring one part by weight of each dentifrice with one or two or three parts by weight of water to obtain dentifrice:water ratios of 1:1, 1:2, and 1:3, respectively. These slurries were mixed on a magnetic stir plate for 20 min and the suspension centrifuged at 10,000 RPM for 10 min. The dentifrice supernatant was removed for tests. For these tests, bacterial cultures (1.9 ml) at optical density of 0.1 at 610 nm were incubated with each prepared dentifrice supernatants (0.1 ml) for 2 min. The treated cultures and untreated controls were rapidly diluted in PBS for viable count determinations on blood agar.

To examine the effects of dentifrice supernatants over time on a range of oral bacteria the dentifrices were prepared by vigorously mixing toothpastes (25 g) with sterile distilled water (50 ml) by

Table 1 The antimicrobial effects of test formulation B (triclosan/copolymer) vs. other formulations. All pastes killed 100% of the organism tested at 60 min (data not shown)

Organism	Strain designation and source	Starting mean log CFU/ml	Mean log CFU/ml after 60 min of treatment			
			Saline	Paste A	Paste B	Paste C
Actinomyces naeslundii	ATCC #19039	7.634±0.075	7.182±0.022	3.799±0.288	2.766±0.176*	5.837±0.300
Actinomyces viscosus	ATCC #43146	7.372±0.006	7.268±0.011	2.426±0.139	0.000±0.000*	2.683±0.076
Capnocytophaga faecalis	ATCC #33709	7.840±0.093	7.316±0.105	6.972±0.151	5.845±0.015*	7.443±0.043
Capnocytophaga gingivalis	ATCC #33624	8.857±0.068	8.084±0.362	5.678±0.029	3.361±0.085*	7.812±0.057
Capnocytophaga ochracea	ATCC #27872	8.224±0.020	7.603±0.091	6.201±0.201	3.321±0.131*	7.264±0.103
Fusobacterium necrophorum	ATCC #27852	8.812±0.053	7.671±0.064	6.854±0.077	5.985±0.056*	7.482±0.127
Fusobacterium nucleatum	ATCC #10953	7.796±0.059	7.674±0.121	6.769±0.018	5.959±0.027*	7.458±0.036
Porphomonas gingivalis	ATCC #49046	8.450±0.020	7.580±0.146	0.000±0.000**	0.000±0.000**	0.000±0.000**
Prevotella intermedia	ATCC #33184	8.153±0.051	7.470±0.226	5.724±0.114	4.298±0.269*	6.342±0.055
Staphylococcus aureus	ATCC #6341	8.898±0.004	8.844±0.024	2.334±0.092	0.301±0.001*	2.393±0.041
Streptococcus mutans	ATCC #25175	8.671±0.168	7.170±0.032	6.311±0.684	2.844±0.241*	7.012±0.544
Veillonella atypica	ATCC #27215	8.753±0.048	7.812±0.122	0.000±0.000**	0.000±0.000**	0.000±0.000**
Veillonella parvula	ATCC #10790	8.360±0.080	7.421±0.009	1.737±0.338	0.545±0.945*	2.384±0.517

* $P \leq 0.001$ vs. other treatments (ANOVA)
** Paste B treatment was not significantly different from Paste A or C treatment at 60 min by ANOVA post hoc testing.

vortexing for 10 min. The mixture was centrifuged at 8,000 *g* for 30 min and the supernatant carefully removed for tests. This aqueous toothpaste supernatant was further diluted 1:20 (v/v) with sterile distilled water and served as the stock solution for all tests of antimicrobial activity with laboratory strains of bacteria. Aliquots (1 ml) of each adjusted bacterial culture were transferred to microfuge tubes and centrifuged at 10,000 *g* for 1 min to sediment the cells. The bacterial pellets were resuspended in 1 ml prereduced PBS plus 1% catalase (control) or 1 ml of one of the three aqueous toothpaste extracts. Aliquots (0.1 ml) from each tube were removed at before treatment and 2, 30, and 60 min after treatment and serially diluted in prereduced PBS plus 1% catalase and plated (Spiral Systems Model DU-2, Spiral Biotech, Bethesda, Md., USA) for viable count determinations on appropriate agar media. The inoculated bacteriological media were incubated in the Coy chamber at 37°C for 5–7 days for anaerobes and at 37°C for 2–3 days for aerobes. Agar plates were scored for bacterial colonies using a CASBA 4 automated plate counter (Spiral Systems) and CFU/ml determined. The procedures with anaerobic bacteria were primarily conducted in the Coy anaerobic chamber and all exposure to unfavorable ambient atmospheric conditions restricted.

Antimicrobial substantivity of dentifrice formulations

The antimicrobial substantivity of the three dentifrices were examined in tests that utilized HA squares as a surrogate for human enamel. The binding and antimicrobial efficacy during subsequent release of the agent from HA were conducted in duplicate for each treatment with each square incubated in aqueous toothpaste extracts (5 ml) and additional HA squares placed in sterile PBS (5 ml) as a control. The squares were incubated with the treatments (2 min) and then washed thrice with 5 ml sterile water. Each HA square was incubated with 1 ml of an overnight culture of *A. naeslundii* grown in Trypticase Soy Broth (adjusted to A_{560}=0.7). An aliquot of the culture (0.1 ml) was immediately removed from all tubes for microbial estimation. All tubes were incubated anaerobically at 37°C and sampled at 1 h for viable bacteria by plating onto agar plates (as described above with a spiral plater). Plates were incubated anaerobically and viable bacterial counts determined for each treatment.

Ex vivo experiments

Human volunteers and oral bacterial sample collection

Samples were collected for ex vivo studies from adult male and female volunteers in good oral and medical health. Subjects on prescription medication or antibiotics were excluded, with all participating subjects undergoing a 15-day washout period prior to sample collection. To comply with the washout procedures volunteers brushed with the provided soft bristled toothbrush and a commercially available fluoride dentifrice and discontinued the use of all other oral hygiene formulations including chewing gums and mints. On the day of sample collection subjects arrived without undertaking any oral care procedures and rinsed their mouths with 10 ml water (Poland Spring Water, Hollis, Me., USA) for 10 s to provide a sample of their oral bacteria. This sample is referred to here as the saliva rinse sample. This sampling method collects bacteria residing in the saliva and also includes bacteria loosely adhering to the teeth.

The saliva rinse samples were preferable to whole saliva for laboratory manipulations, since these samples were of uniform consistency. Subjects tongues were scraped with a sterile brush (MasterAmp Buccal Swab Brushes, Epicenter, Madison, Wis., USA) and these scrapings were pooled with their saliva rinse samples prior to tests. Eleven subjects participated in this study to examine the effects of dentifrices ex vivo (eight men, 3 women; mean age 43.3 years, median 44, range 23–54).

Microbiological procedures with human oral samples

The ex vivo test procedures examined the effects of paste A and B. Test dentifrices were prepared by weighing out approximately 10 g in a 250 ml beaker to which was added an equal weight of distilled water. The beakers were stirred at room temperature for 20 min and the slurry centrifuged at 10,000 rpm for 10 min to obtain supernatants that were decanted for tests. Subjects provided a pooled sample of saliva rinse and tongue scrapings. Aliquots of these pooled oral bacterial samples from each subject (1.9 ml) were mixed with 0.1 ml of each test dentifrice preparation and incubated for 2 min at room temperature. In addition to the dentifrice-treated samples, an untreated control sample was then diluted in tenfold dilutions in PBS with 0.1 ml of all dilutions (dentifrice treated and control) plated in duplicate on blood agar to enumerate all cultivable oral bacteria and on an oral hydrogen-sulfide producing agar to detect oral bacteria producing H_2S respectively. All plates were incubated anaerobically at 37°C for 5–7 days in accordance with standard procedures [5, 10] and colony forming units from each medium determined. Average numbers of bacteria per subject and sampling condition in CFU/ml were transformed to $\log_{10}$ and are reported as average log CFU/ml.

Statistical analysis

For all tests average bacterial CFU/ml were determined and transformed to $\log_{10}$ prior to statistical analysis. To examine the effect of test product concentrations on bacteria a two-way analysis of variance (ANOVA) was conducted (with concentration and test product as the two factors) using the SAS software (SAS Institute, Cary, N.C., USA) with post-hoc comparisons conducted by the Tukey test. One-factor ANOVA was utilized to examine the effects of test products on bacterial species over time with post-hoc analysis (Fisher's and Scheffe's S tests) conducted when significant differences were noted by ANOVA. For the ex vivo tests *t* test analyses were conducted with the Microsoft Excel software. Statistical significance for all tests was set at $P<0.05$.

Results

Dose-dependent effects of dentifrices

The effects of different doses of test dentifrices were examined with *A. viscosus* as a model system. Figure 1 describes the average results from triplicate samples expressed as a percentage of the initial bacterial concentration added to each test. Statistically significant differences between paste B and the control dentifrices tested (formulations A and C) were noted at each concentration of triclosan tested ($P<0.05$). Additionally, post-hoc comparisons indicate a significant dose-response effect at different concentrations of triclosan/copolymer (paste B; $P<0.05$). In contrast, a dose response was not evident with the control fluoride dentifrice formulations (pastes A and C).

In vitro antimicrobial efficacy over time

When the three formulations were compared to the saline control, each was seen to reduce microbial growth over the 60-min period. However, the triclosan/copolymer formulation (paste B) was significantly superior to the other formulations in 11 of 13 species tested at the 60 min

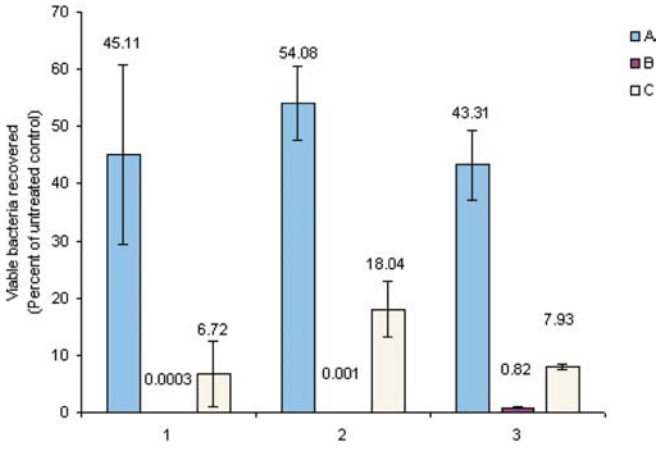

Fig. 1 The dose-dependent effect of three test dentifrices on *Actinomyces viscosus*. Dentifrices were diluted and concentrations 1, 2, and 3 correspond to 50, 37.5, and 30 μg/ml of triclosan applied for dentifrice B. Results indicate average viable bacteria recovered after each treatment as a percentage of untreated control (also indicated as data labels) ± standard error. Paste B demonstrated a statistically significant ($P<0.05$) effect vs. all other pastes at each of the three dentifrice concentrations tested by two-way ANOVA. Paste B also shows a significant dose-response effect ($P<0.05$) in contrast to the other pastes

time point ($P<0.05$). In the case of *F. nucleatum* and *C. gingivalis* kill curves (Figs. 2, 3) demonstrate significant killing by paste B at all time points compared to formulations A and C ($P<0.05$). With *P. intermedia* (Fig. 4) significant effects were noted by formulation B at 30 and 60 min of exposure ($P<0.05$) and with *A. naeslundii* at 60 min (Fig. 5; $P<0.05$). A summary of the effects of formulation B on all bacteria at 60 min in comparison to controls is shown in Table 1.

Substantivity of efficacy from HA squares

The substantive antimicrobial effects of the three test dentifrices is shown in Fig. 6 with *A. naeslundii* as a model system representing bacterial strains sensitive to triclosan/copolymer (paste B). In the above in vitro antimicrobial efficacy test over time, formulations A and C had some effect at the 60 min time point in the case of *A. naeslundii*. Whereas immediately posttreatment no antimicrobial effects were noted with any dentifrices, at 60 min posttreatment dentifrice B was the only treatment that resulted in a significant reduction in viable bacteria ($P<0.05$). The effects by the other dentifrices (A and C) at 60 min posttreatment were comparable to the saline treated controls and indicate an absence of residual antimicrobial effects. Therefore the results indicate the ability of paste B (triclosan/copolymer) to bind to HA and provide residual antimicrobial effects on bacteria over time in order to inhibit microbial viability.

Effects of dentifrice treatments on human oral bacterial samples ex vivo

Eleven adult subjects participated in a study to examine the effects of treating bacteria obtained from the human mouth (obtained by rinsing to which were also added tongue scrapings) by the two test dentifrices (A and B). Paste C was not tested due to its low activity noted in

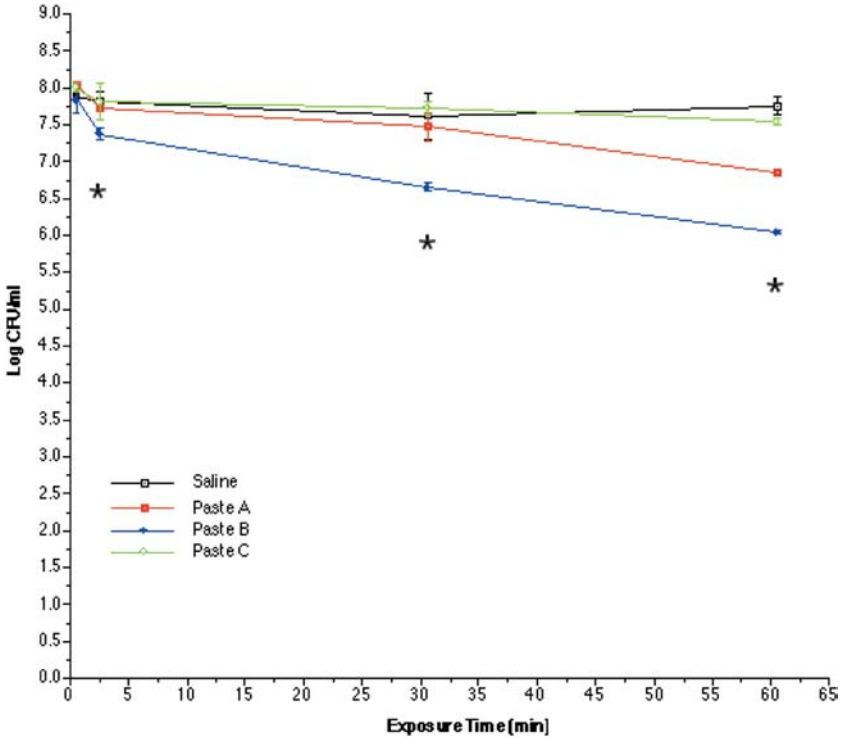

Fig. 2 The effect of test dentifrices on *F. nucleatum* over time. Note that paste B shows a significantly greater effect 2, 30, and 60 min after treatment. Results indicate average viable bacteria recovered at each sampling point (log CFU/ml) ± standard deviation. *$P<0.05$ reduction in log CFU/ml vs. control dentifrices (ANOVA)

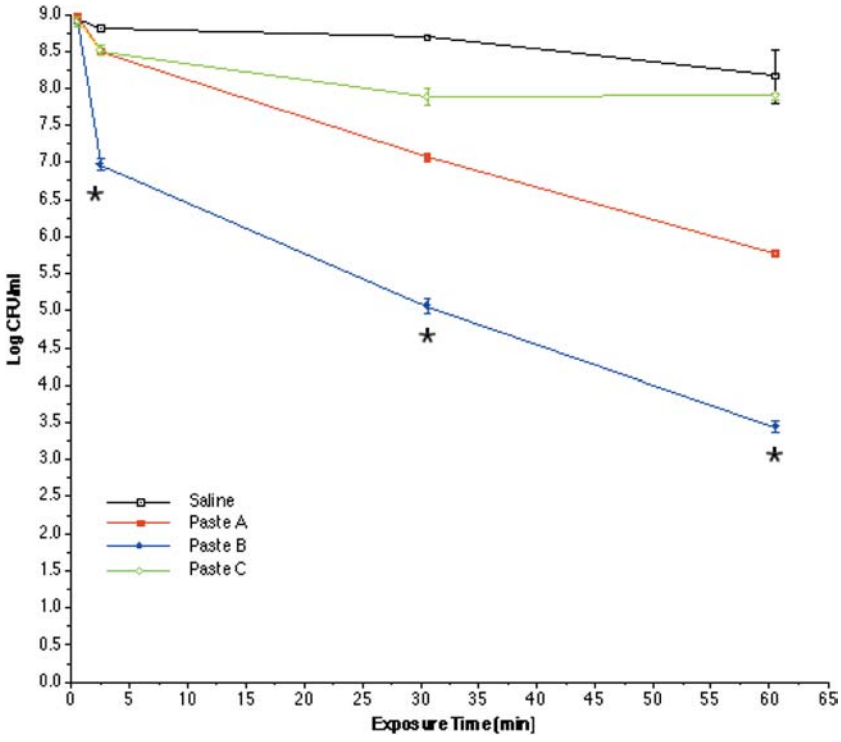

Fig. 3 The effect of test dentifrices on *C. gingivalis* over time. Note that paste B shows a significantly greater effect 2, 30, and 60 min after treatment. Results indicate average viable bacteria recovered at each sampling point (log CFU/ml) ± standard deviation. *P<0.05 reduction in log CFU/ml vs. control dentifrices (ANOVA)

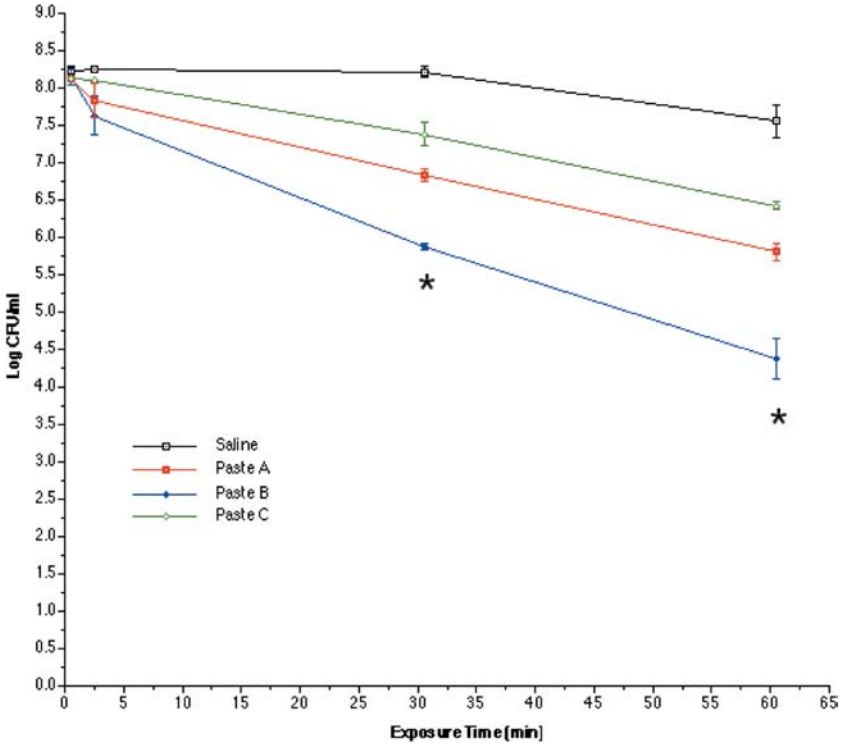

Fig. 4 The effect of test dentifrices on *P. intermedia* over time. Note that paste B shows a significantly greater effect 30 and 60 min after treatment. Results indicate average viable bacteria recovered at each sampling point (log CFU/ml) ±standard deviation. *P<0.05 reduction in log CFU/ml vs. control dentifrices (ANOVA)

previous tests. Figure 7 presents the average numbers of each type of bacteria isolated (log CFU/ml) after a 2-min incubation with dentifrices A and B. Treatment with paste B resulted in a statistically significant reduction in cultivable bacteria by 90% and the H_2S producing by 92% vs. paste A (P<0.05).

Discussion

Triclosan/copolymer is effective against both Gram-positive and Gram-negative bacteria with recent clinical results indicating effects on dental plaque bacteria. For instance, triclosan/copolymer reduces the microbial vitality of supragingival plaque [1] and provides a qualitative and

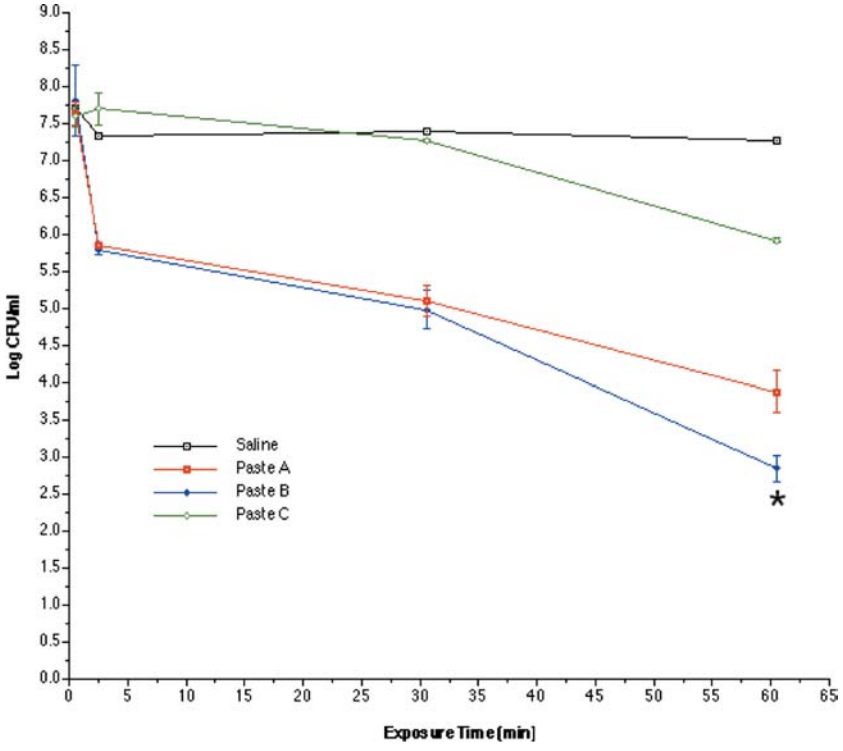

Fig. 5 The effect of test dentifrices on *A. naeslundii* over time. Note that paste B shows a significantly greater effect 60 min after treatment. Results indicate average viable bacteria recovered at each sampling point (log CFU/ml) ± standard deviation. *P<0.05 reduction in log CFU/ml vs. control dentifrices (ANOVA)

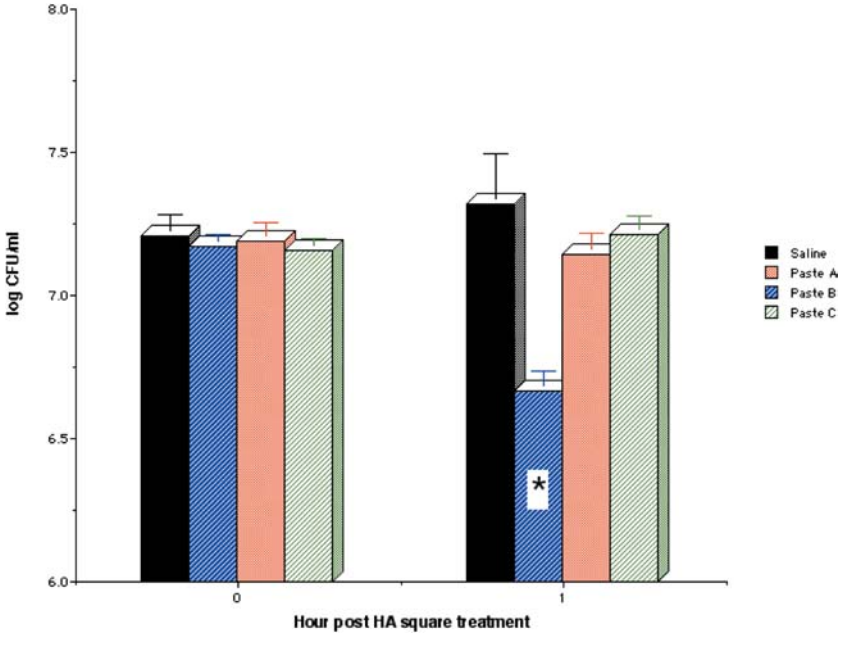

Fig. 6 The antimicrobial substantivity of paste B (triclosan/copolymer) and control dentifrices on *A. naeslundii*. *Left* No release of active agents immediately from the hydroxyapatite squares: *right* release of active agents after 1 h. Paste B (triclosan/copolymer) is the only formulation that shows antibacterial activity in this assay. Results indicate average viable bacteria recovered at each sampling point ± standard deviation. *P<0.05 reduction in log CFU/ml vs. control dentifrices (ANOVA)

quantitative decrease in subgingival microflora following extended use [15]. The significant reductions in organoleptic scores shown by the triclosan/copolymer dentifrice [17, 21] coupled with its effects on dental plaque [5, 20, 21] formed the basis of these microbiological studies. These studies were conducted to help explain the efficacy of this dentifrice on oral malodor in comparison to commercially available dentifrices.

The microbiology of halitosis has been investigated by three principal approaches: in vitro laboratory susceptibility assays, substantivity assays and ex vivo studies. In each approach efforts were made to simulate conditions that were representative of those seen in the oral cavity. Antimicrobial test procedures were rigorously monitored with reference to concentrations of test dentifrices, exposure periods, and numbers of bacteria to mimic conditions in the human mouth following routine dentifrice use. These areas were initially addressed in studies that explored a range of test dentifrice concentrations. Experiments with *A. viscosus* examined the antimicrobial

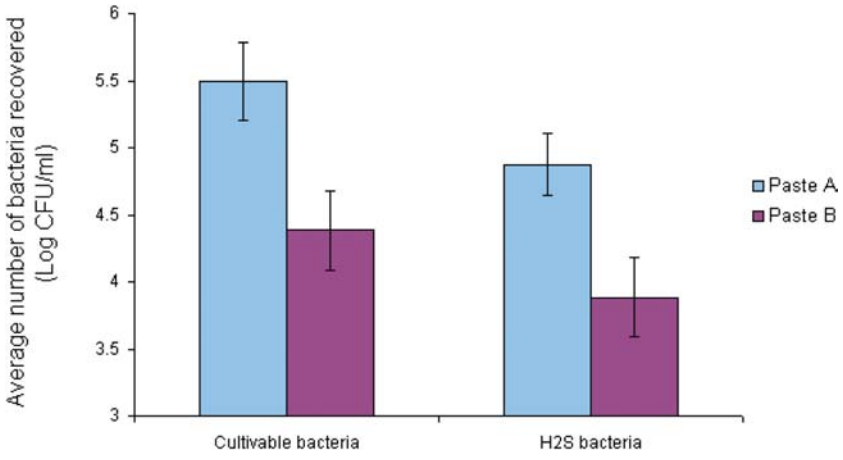

Fig. 7 Ex vivo effects of dentifrices on oral bacteria from human volunteers on cultivable bacteria and those producing H_2S. Shown are the average number of bacteria recovered (Log CFU/ml) ± standard error after treatments with paste A and B. *P<0.05 reduction in bacteria by paste B vs. paste A (control dentifrice)

effects of 30–50 μg/ml (or ppm) of triclosan. A dose-dependent effect was noted with triclosan/copolymer reflecting a fundamental antimicrobial effect. In contrast, a dose response was not evident with the control dentifrices at various concentrations.

Subsequent in vitro kill experiments and ex vivo studies utilized the above concentrations of triclosan in the applied dose for tests. Microbial genera chosen for testing included: *Prevotella*, *Porphyromonas*, *Veillonella*, *Capnocytophaga*, and *Fusobacteria*. These are bacteria commonly associated with halitosis. Bacterial concentrations for the in vitro tests were based on previous clinical results [4] in which plaque collected from over 50 subjects from 20 teeth yielded a wet weight of 11–12 mg per patient. Since sampling two teeth yields approx. 1 mg wet weight dental plaque that corresponds to approx. 1×10^7 micro-organisms per milligram, the plaque from 20 teeth should yield approx. 1×10^8 which formed the bacterial concentrations in a majority of in vitro tests. For the ex vivo test the dentifrice dose was added to a starting concentration of approx.1×10^7 bacteria per milliliter obtained from the saliva and tongue. In most mouthrinse studies the bacteria found in saliva range from $0.1–1\times10^6$ per milliliter of saliva. For the tests in this study tongue scrapings were also added to salivary bacteria which led to a higher level of bacteria captured in the salivary oral rinse collected. Thus the concentrations chosen for these experiments were based on expected relationships between the dose of triclosan and concentrations of targeted bacteria in the human mouth.

In the in vitro tests, after a 2 min exposure, the significant effects of the triclosan/copolymer dentifrice were noted on 11 of the 13 strains examined. The modest initial antimicrobial effects of exciepients in oral care formulations (such as flavors and surfactants) is well known [3]. However, effects over time (60 min posttreatment) were significantly higher with the triclosan/copolymer paste than the control dentifrices. With a few strains the control dentifrices demonstrated activity at 60 min when bioavailable in solution. Residual antimicrobial activity of dentifrice agents found in the mouth after brushing is an important attribute if longitudinal effects are expected [3]. Therefore a substantivity test was conducted 60 min after dentifrice treatment demonstrating dentifrice bound and released from HA squares and confirming the significant residual efficacy of the triclosan/copolymer dentifrice. These results corroborate previous clinical studies that indicate a higher substantivity of triclosan upon formulation with copolymer [7].

The inclusion of clinical strains for antimicrobial tests has been increasingly recommended [18]. A procedure for the collection of oral samples was standardized to incorporate the complex oral microflora from anatomical sites in the human mouth implicated in malodor [9] and included tongue scrapings for bacteria in the biofilm mode of growth. As seen with laboratory strains, the triclosan/copolymer dentifrice was significantly effective on these clinical oral bacterial samples including those producing H_2S and implicated in malodor [10].

The unique physiology of the human mouth most likely poses a clear challenge for the delivery and activity of therapies for the inhibition of malodor. For example, the mouth with its distinct niches that include mucosal surfaces and tooth surfaces as well as salivary flow, along with dietary factors, all contribute to the significant changes seen in the environment during the course of the day. In addition to these changing conditions of the mouth, the presence of large numbers of bacteria that are localized in specific areas of the mouth also contribute to the modest effects noted on malodor in clinical studies when fluoride dentifrices are utilized [9, 21]. Previous clinical studies report the significant effects of the triclosan/copolymer dentifrice in organoleptic parameters [17, 21]. Taken together, the results from this present investigation and previous clinical trials demonstrating significant reductions in organoleptic scores indicate that the mitigation of breath odor by the triclosan/copolymer formulation may be explained in some measure by the antimicrobial effects of this dentifrice.

Acknowledgement Statistical analysis by Larni Espada, Technology Statistics, Colgate-Palmolive Company, is gratefully acknowledged.

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